Variability of Directly Measured First-Pass Hepatic Insulin Extraction and Its Association With Insulin Sensitivity and Plasma Insulin

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Although the β-cells secrete insulin, the liver, with its first-pass insulin extraction (FPE), regulates the amount of insulin allowed into circulation for action on target tissues. The metabolic clearance rate of insulin, of which FPE is the dominant component, is a major determinant of insulin sensitivity (SI). We studied the intricate relationship among FPE, SI, and fasting insulin. We used a direct method of measuring FPE, the paired portal/peripheral infusion protocol, where insulin is infused stepwise through either the portal vein or a peripheral vein in healthy young dogs (n = 12). FPE is calculated as the difference in clearance rates (slope of infusion rate vs. steady insulin plot) between the paired experiments. Significant correlations were found between FPE and clamp-assessed SI (r_s = 0.74), FPE and fasting insulin (r_s = −0.64), and SI and fasting insulin (r_s = −0.67). We also found a wide variance in FPE (22.4–77.2%; mean ± SD 50.4 ± 19.1) that is reflected in the variability of plasma insulin (48.1 ± 30.9 pmol/L) and SI (9.4 ± 5.8 × 10^4 dL · kg^{-1} · min^{-1} · [pmol/L]$^{-1}$). FPE could be the nexus of regulation of both plasma insulin and SI.

Plasma insulin is determined by pancreatic β-cell secretion and metabolic clearance, which is the aggregate catabolism of insulin by all insulin-sensitive tissues. The liver extracts up to 80% of secreted insulin (1) during passage through the portal vein (first-pass extraction [FPE]), controlling the amount accessible to the periphery for action on intended extrahepatic tissues. Hyperinsulinemic compensation during the induction of insulin resistance is the combination of both increased insulin secretion and reduced FPE (2,3). During persistent insulin resistance, however, the reduction in FPE primarily sustains the hyperinsulinemic compensation (2,3), yet less attention has been focused on FPE than on β-cell function in the determination of plasma insulin. Rodent studies have shown that inhibition of hepatic insulin extraction causes hyperinsulinemia, insulin resistance, and attendant disorders (4,5). African Americans, for example, have reduced FPE (6), elevated plasma insulin (7), low insulin sensitivity (SI) (7), and a high risk of metabolic diseases compared with Caucasians (8,9). These reports have supported the essentiality of FPE in insulin resistance and associated metabolic diseases and suggested that more attention be focused on FPE. Low FPE could be a major risk factor for insulin resistance. Treatment of high-risk individuals (low FPE) with appropriate interventions could be vital to controlling insulin resistance and its associated comorbidities. Direct measurement of FPE is difficult because deep-seated vessels must be cannulated; therefore, clinical studies often use surrogate methods. By using a large animal model, the canine, which enables us to measure hepatic insulin extraction directly, we investigated the relationship among FPE, SI, and plasma insulin in a sample of healthy animals.

RESEARCH DESIGN AND METHODS

Animal Care

Under the oversight of the Cedars-Sinai Medical Center Institutional Animal Care and Use Committee, study animals were housed under a 12-h light, 12-h dark cycle in a temperature-controlled vivarium where they were inspected daily by board-certified veterinarians. The dogs were fed a standard diet comprising one can of Purina Pro Plan Puppy Chow (10% protein, 7% fat, 1.5% fiber, and 76% moisture; Nestlé Purina Petcare, St. Louis, MO) and 825 g dry chow (2.9% fiber, 27.7% protein, 29.9% fat, and 42.4% carbohydrate

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[LabDiet; PMI Nutrition, Brentwood, MO]) for a total of 3,576 calories/day (39.2% carbohydrate, 32.5% fat, and 28.3% protein). The study animals always had access to water.

Surgery

Twelve male mongrel dogs ~1 year old underwent laparotomic surgery, and 7-F catheters were inserted ~3.5 cm distal from the porta hepatis and secured in place with 5-0 Prolene suture. The catheter was then tunneled subcutaneously and connected to a planted vascular access port at the iliocostalis lumborum muscle. Liver biopsy specimens were taken and snap frozen for molecular assessments. The abdominal cavity was sutured with 2-0 Monocryl sutures. The dogs were allowed 2–3 weeks to recover and achieve steady body weight. The vascular access port and catheter were kept patent by locking with 2.0 mL taurolidine-citrate catheter solution (Access Technologies, Skokie, IL). Experiments were carried out after an overnight fast, and before each study, body temperature and hematocrit were checked.

Experiments

Three randomized experiments were performed on each of the 12 dogs in the conscious state. The experiments were separated by at least 3 days. The portal and peripheral insulin infusion (PPII) protocols were paired experiments and, as such, were performed consecutively, separated by the recovery days.

Hyperinsulinemic-Euglycemic Clamp

At $t = -120$ min, a primed infusion (25 μCi + 0.25 μCi/min) of [3-^{3}H]glucose (PerkinElmer, Waltham, MA) to estimate glucose turnover was started and continued throughout the experiment. After 90 min of tracer equilibration, four basal samples were taken 10 min apart from $t = -30$ min. After the last basal sample at $t = 0$ min, a continuous peripheral infusion of 4.5 pmol/kg/min porcine insulin (Eli Lilly, Indianapolis, IN) to sustain hyperinsulinemia and 1.0 μg/kg/min somatostatin (Bachem, Torrance, CA) to inhibit insulin and glucagon secretion was started and maintained until the end of the experiment. To maintain euglycemia, a variable rate infusion of 50% (454 mg/mL) dextrose (glucose infusion [GINF]) mixed with [3-^{3}H]glucose for a specific activity of 2.0 μCi/g was adjusted as necessary. Blood samples were taken every 10 min from $t = 0$–60 min, every 15 min from $t = 60$–120 min, and every 10 min from $t = 120$–180 min. The period of 150–180 min was considered the steady state.

Paired PPII Protocol

After three basal samples were taken at the $t = -110$, -100, and -91 min, 1.0 μg/kg/min somatostatin was started at $t = -90$ min through a saphenous vein and throughout the experiment to inhibit insulin secretion during either the portal or the peripheral insulin infusion protocol (Fig. 1). Beginning at $t = 0$ min, replacement glucagon at 1.3 ng/kg/min was infused into the portal vein and continued throughout the experiment during both portal and peripheral insulin infusion protocols. Plasma glucose was clamped at the measured basal concentration during the experiments by a variable rate GINF through a saphenous vein.

Peripheral Insulin Infusion During Clamp

Starting at $t = 0$ min, insulin was infused at three successive incremental rates, 1.5, 3.0, and 4.5 pmol/kg/min, through a peripheral vein. Each infusion rate lasted 90 min, of which the last 30 min was considered the steady state. Blood samples were taken every 10 min from another peripheral vein for the assays.

Portal Insulin Infusion During Clamp

Through the portal vein, insulin was infused at three successive incremental rates (two times the rate of peripheral infusion): 3.0, 6.0, and 9.0 pmol/kg/min. Each infusion rate spanned 90 min, and the last 30 min of each rate was considered the steady state. Blood samples were taken every 10 min from a peripheral vein. Rates twice the insulin infusion rates of the peripheral clamp experiments were used for the portal protocol, with the aim of achieving matching circulating plasma insulin between the two experiments on the assumption that the liver extracts ~50% of portal insulin (10). The infusion rates used cover the physiological ranges of plasma insulin from fasting to postprandial.

Sample Collection and Glucose, Insulin, and Tracer Assays

One milliliter of blood was taken at each time point from a peripheral vein into chilled 1.5-mL Eppendorf tubes coated with lithium fluoride, heparin, and 1 μg/50 μL EDTA. Collected samples were immediately centrifuged, plasma was aliquoted, and glucose concentrations were measured with a YSI 2700 autoanalyzer (Yellow Springs Instruments, Yellow Springs, OH). The rest of the plasma samples were stored at −20°F until ready for insulin measurements and [3-^{3}H]glucose tracer assay in the case of the hyperinsulinemic-euglycemic clamp (EGC). A sandwich ELISA kit designed for porcine and canine insulin (80-INSPO-E01; ALPCO, Salem, NH) was used to assay the insulin. The ELISA has an identical sensitivity to porcine and canine insulin. The intra- and interassay coefficient of variance of insulin was 2.3 ± 0.3% and 2.9 ± 1.3%, respectively. Samples were processed on ice. Average baseline insulin measurements of each dog before the experiments were considered the fasting insulin level. The plasma samples from the EGC were processed according to Ader and Bergman (11), and the specific activity of the [3-^{3}H]glucose tracer was assayed by a liquid scintillation counter (LS 6000SC; Beckman Coulter, Fullerton, CA).

Liver Function Assays

The liver function panels were assayed from fasting blood samples by ANTECH Diagnostics (Irvine, CA), a veterinary laboratory service provider.

Total RNA Isolation and Gene Expression Assays

A TRI Reagent kit (Molecular Research Center, Cincinnati, OH) was used for the extraction of total RNA from the liver

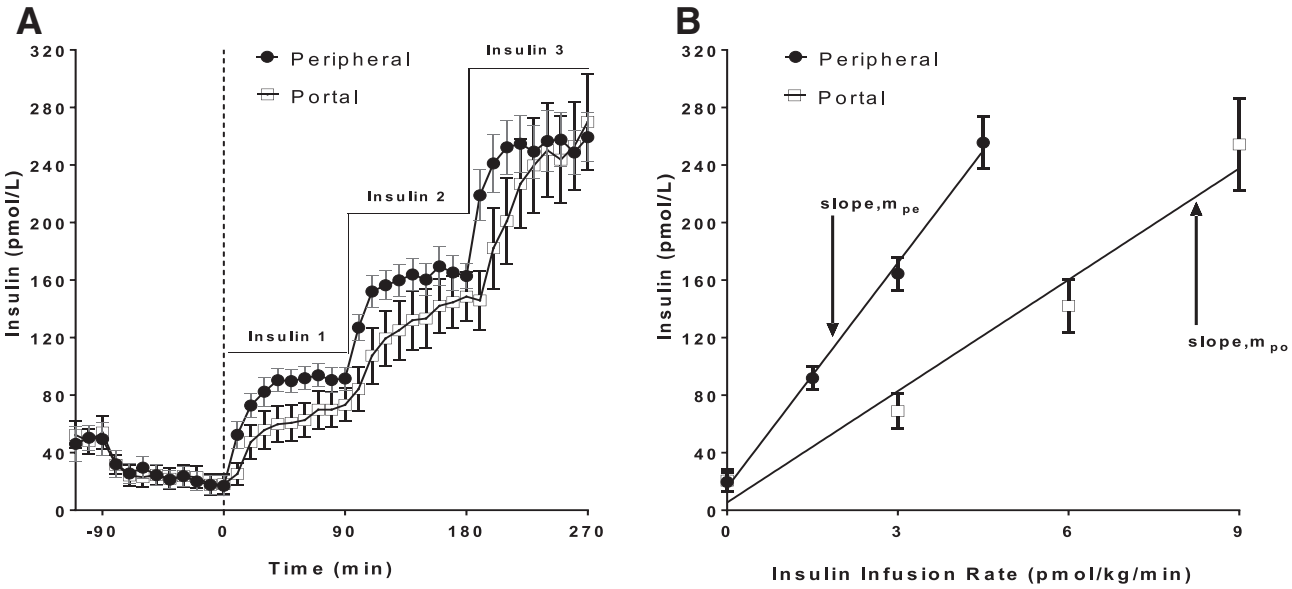

Figure 1—PPII method for measuring FPE. *A*: Insulin profile during the PPII experiments. Insulin 1, 3.0 and 1.5 pmol/kg/min intraportal and peripheral infusions, respectively. Insulin 2, 6.0 and 3.0 pmol/kg/min intraportal and peripheral infusions, respectively. Insulin 3, 9.0 and 4.5 pmol/kg/min intraportal and peripheral infusions, respectively. *B*: Infusion rate versus steady-state plasma insulin with slope m. For portal and peripheral infusion, respectively, r_s = 0.98 and 0.99. Slope of the portal infusion plot (m_{po}) was 25.8 kg * min/mL and that of the peripheral infusion (m_{pe}) was 52.0 kg * min/mL for n = 12. FPE (%) = [1 − (m_{po}/m_{pe})] * 100 = 50.4%; for derivation of equation refer to calculations in RESEARCH DESIGN AND METHODS.

biopsy samples according to the accompanying protocol. One microgram of the total RNA was reversed transcribed by the SuperScript First-Strand Synthesis System for RT-PCR kit (Invitrogen, Carlsbad, CA) to first-strand cDNA. Predesigned TaqMan real-time PCR assays (insulin receptor [INSR], Cf02647625_m1; insulin-degrading enzyme [IDE], Cf02634270_m1; carcinoembryonic antigen–related cell adhesion molecule 1 [CEACAM1], Cf03054097_m1; and 18*s* rRNA, Hs99999901_s1 [Applied Biosystems, Foster City, CA]) with a LightCycler 4.8 instrument (Roche Life Science, Indianapolis, IN) were used to assess gene expression. Data were analyzed by the relative quantification method by using the 18*s* rRNA gene as the internal control for normalization.

Calculations

SI was calculated from the EGC (Eq. 1):

$$SI = \left(\frac{\Delta GINF}{\Delta Ins * Glc}\right) \quad \text{(Eq. 1)}$$

where ΔGINF is GINF rate at steady state normalized by body weight – GINF rate at basal normalized by body weight, ΔIns is plasma insulin concentration at steady state – plasma insulin at basal, and Glc is glucose concentration at steady state.

From the infused tracer during the EGC, we also calculated hepatic SI (SiHGO) (Eq. 2) and peripheral SI (SiP) (12,13) (Eq. 3):

$$SiHGO = \left|\frac{\Delta HGO}{\Delta Ins * Glc}\right| \quad \text{(Eq. 2)}$$

$$SiP = \frac{\Delta Rd}{\Delta Ins * Glc} \quad \text{(Eq. 3)}$$

Hepatic glucose production (HGO) and peripheral glucose uptake (Rd) were calculated from the modified Steele equation (14), where ΔHGO (suppression of glucose production) and ΔRd (increasing peripheral glucose uptake) are the respective changes from basal to steady state during the EGC.

FPE from the paired PPII protocol was calculated according to the isotopic dilution principle (1) (Eq. 4a):

$$CL\ (mL/kg/min) = \frac{\text{insulin infusion rate}\ \ (pmol/kg/min)}{\text{steady-state plasma insulin conc.}\ (pmol/L)} \quad \text{(Eq. 4a)}$$

Assuming linear insulin kinetics within the concentration studied, insulin clearance rate (CL [mL/kg/min]) can thus be calculated as the inverse of the slope (m) of the least squares regression line between insulin infusion rate (pmol/kg/min) and steady-state plasma insulin concentration (pmol/L) (3,11). Hence CL can be expressed as in Eqs. 4b and 5:

$$CL\ (mL/kg/min) = \frac{1}{m\ (kg * min/mL)} \quad \text{(Eq. 4b)}$$

$$\text{FPE CL } (\text{mL/kg/min}) = \text{CLpo} - \text{CLpe} \qquad \text{(Eq. 5)}$$

where CLpo and CLpe are insulin clearance rates during the portal infusion and peripheral infusion protocols, respectively. Equation 6 shows the calculation for percent FPE:

$$\text{FPE } (\%) = \left(\frac{\text{CLpo} - \text{CLpe}}{\text{CLpo}}\right) * 100 \qquad \text{(Eq. 6)}$$

Substituting Eq. 4b into Eq. 6 gives Eq. 7:

$$\text{FPE } (\%) = \left(1 - \left(\frac{\text{mpo}}{\text{mpe}}\right)\right) * 100 \qquad \text{(Eq. 7)}$$

where mpo and mpe are the respective slopes of portal infusion rate versus steady-state plasma insulin and peripheral infusion rate versus corresponding steady-state plasma insulin. In analyzing the distribution of FPE, we include the baseline assessments of 6 dogs from a previous study (3) that used the same experimental protocol for FPE measurements as used for the current 12 to increase the sample size.

Statistics

Spearman rank order correlation (r_s) is used for the association analysis. $P < 0.05$ is the set level of significance. All data are reported as mean ± SEM unless stated otherwise.

RESULTS

The average body weight of the 12 dogs was 27.5 ± 1.0 kg. Mean fasting insulin was 48.1 ± 8.9 pmol/L, spanning 23.3–118.4 pmol/L, a 5.1-fold range, and fasting glucose was 95.3 ± 1.4 mg/dL, indicating no glucose dysregulation. Two dogs had high fasting insulin concentrations of 104.4 and 118.4 pmol/L. For one of the two dogs, the fasting insulin concentrations on three different days (each with three or more samples taken) were 108.9, 126.1, and 120.2 pmol/L, whereas those of the other dog were 113, 101.6, and 98.7 pmol/L. The consistent high insulin levels indicate that they were not spurious measurements. The mean basal HGO was 2.0 ± 0.1 mg · kg^{-1} · min^{-1}, spanning 1.2–2.5 mg · kg^{-1} · min^{-1}, a 2.1-fold range. Mean SI was 9.4 ± 1.7 × 10^4 dL · kg^{-1} · min^{-1} · $(pmol/L)^{-1}$, spanning 3.0–26.2 × 10^4 dL · kg^{-1} · min^{-1} · $(pmol/L)^{-1}$, an 8.8-fold range. The mean SiHGO was 2.7 ± 0.6 × 10^4 dL · kg^{-1} · min^{-1} · $(pmol/L)^{-1}$, spanning 1.1–8.7 × 10^4 dL · kg^{-1} · min^{-1} · $(pmol/L)^{-1}$, a 7.7-fold range, whereas the mean SiP was 6.7 ± 1.2 × 10^4 dL · kg^{-1} · min^{-1} · $(pmol/L)^{-1}$, spanning 1.7–18.0 × 10^4 dL · kg^{-1} · min^{-1} · $(pmol/L)^{-1}$, a 10.6-fold range. FPE spanned 22.4–77.2%, a 3.5-fold range (Table 1). Liver function panels were normal (Table 2). With consideration of the wide range of FPE realized, we added the baseline data of six dogs from a previous study (3) to increase the sample size as we assessed the variability of FPE in normal dogs. For the 18 dogs analyzed for the variability of FPE, mean FPE was 53.4% (SD 16.5) (Fig. 6).

Correlations

We found significant correlations between FPE and SI (r_s = 0.74; P = 0.006) (Fig. 2*A*), FPE and fasting plasma insulin (r_s = −0.64; P = 0.03) (Fig. 2*B*), and SI and fasting insulin (r_s = −0.67; P = 0.02) (Fig. 2*C*). We then omitted the data of the two dogs with outlier high fasting insulin concentrations and reassessed the correlations among FPE, SI, and fasting insulin; the correlations remained significant and stronger (FPE vs. SI: r_s = 0.82 [P = 0.004]; FPE vs. fasting insulin: r_s = −0.78 [P = 0.008]; SI vs. fasting insulin: r_s = −0.78 [P = 0.008]). We further analyzed the correlations between FPE and the components of SI (SiHGO and SiP) by using the data of all 12 animals. We found a significant correlation between FPE and SiP (r_s = 0.80; P = 0.002) (Fig. 3*A*) and a similar relationship between FPE and ΔRd, from which SiP was defined (r_s = 0.75; P = 0.005). However, we found an insignificant correlation between FPE and SiHGO (r_s = 0.31; P = 0.33) (Fig. 3*B*) and a corresponding relationship between FPE and $|\Delta\text{HGO}|$, from which SiHGO was calculated (r_s = 0.34; P = 0.28).

IDE and CEACAM1 are key proteins involved in hepatic insulin catabolism after insulin is bound to its receptors (4,10,15). We assessed how the expressions of these genes relative to 18s rRNA (arbitrary unit) are associated with FPE. We found a significant association between INSR and FPE (r_s = 0.67; P = 0.02) (Fig. 4*A*). However, IDE and CEACAM1 did not significantly correlate with FPE (CEACAM1 vs. FPE: r_s = 0. 47 [P = 0. 12] [Fig. 4*B*]; IDE vs. FPE: r_s = 0. 31 [P = 0. 33] [Fig. 4*C*]). We also did not find a significant correlation between HGO and INSR (r_s = 0.21; P = 0.51) (Fig. 5*A*). The correlation between FPE and basal HGO was 0.50 (P = 0.10) (Fig. 5*B*).

In addition, no significant correlation was found between FPE and fasting plasma alanine aminotransferase or aspartate aminotransferase. These aminotransferases are used as measures of liver health (16). Similarly, no correlation was realized between FPE and fasting albumin and total bilirubin, clinical surrogates of hepatic function (16).

DISCUSSION

Plasma insulin concentrations are used regularly as the gauge for SI (2,17). Although insulin is secreted by the pancreas, the liver determines how much insulin is allowed into the general circulation for its eventual action on target tissues. Studies have suggested a cross talk between insulin secretion and hepatic insulin extraction in ensuring adequate insulinemia to maintain normal glucose tolerance (3,18). During insulin resistance, which can be a regular physiological change, such as in pregnancy (19), puberty (20), and old age (21), or a pathophysiological condition, such as obesity (2), a combination of increased secretion and reduced hepatic extraction result in hyperinsulinemia to compensate for the reduced insulin action (2,3).

However, decreased hepatic insulin clearance is postulated to cause hyperinsulinemia, which in turn causes

Table 1—FPE, fasting insulin, fasting HGO, whole-body SI, SiP, and SiHGO of dogs

	FPE (%)	Fasting insulin (pmol/L)	Fasting HGO ($mg \cdot kg^{-1} \cdot min^{-1}$)	SI $\times 10^{-4}$ ($dL \cdot kg^{-1} \cdot min^{-1} \cdot [pmol/L]^{-1}$)	SiP $\times 10^{-4}$ ($dL \cdot kg^{-1} \cdot min^{-1} \cdot [pmol/L]^{-1}$)	SiHGO $\times 10^{-4}$ ($dL \cdot kg^{-1} \cdot min^{-1} \cdot [pmol/L]^{-1}$)
Dog 1	22.37	57.0	1.22	6.95	4.02	2.43
Dog 2	24.33	38.85	1.44	2.99	1.70	1.14
Dog 3	31.82	39.13	1.87	7.19	5.59	1.60
Dog 4	39.24	41.65	2.19	9.40	6.85	2.16
Dog 5	42.49	104.43	2.29	4.64	2.64	1.87
Dog 6	44.96	28.47	2.03	10.43	5.54	4.55
Dog 7	53.02	118.40	1.80	8.75	7.06	1.96
Dog 8	58.95	33.12	2.57	7.31	6.13	1.27
Dog 9	66.05	27.27	2.54	9.80	7.51	2.40
Dog 10	71.92	32.96	2.46	8.33	6.73	2.15
Dog 11	72.05	23.27	1.96	26.23	18.0	8.69
Dog 12	77.17	32.45	2.0	10.44	8.50	2.16
Mean ± SEM	50.36 ± 5.51	48.08 ± 8.93	2.03 ± 0.12	9.37 ± 1.67	6.69 ± 1.18	2.70 ± 0.60

insulin resistance (4,22). As such, decreased hepatic insulin extraction is purported to be the cause of insulin resistance rather than a reaction thereof (22). Thus, considerable attention must be focused on FPE, the gatekeeper of plasma insulin, as much as on β-cell function in the determination of the systemic levels of the hormone and SI. The significant correlations among FPE, SI, and fasting insulin (Fig. 2) found in this cross-sectional study, although they do not explain whether reduced FPE is the cause or consequence of insulin resistance, suggest a role of FPE as the nexus for the regulation of plasma insulin level and SI.

FPE controls plasma insulin. Metabolic clearance of insulin, which includes FPE, is a strong determinant of SI (12). Both insulin extraction and insulin action are mediated by identical receptors, which could possibly explain the correlation between FPE and SI. SiHGO and SiP account for whole-body SI, and the relationship between FPE and SI ($r_s = 0.74$; $P = 0.006$) (Fig. 2*A*) seems to be driven predominately by SiP and not so much by SiHGO because of the strong association between FPE and SiP (Fig. 3*A*) and the poor correlation between FPE and SiHGO (Fig. 3*B*). In fact, during EGC, insulin's action at the periphery in stimulating glucose uptake into skeletal muscles and adipose tissue is more expansive than its action on liver (11). The dissociation of FPE and HGO/SiHGO could stem from the differential expressions and

Table 2—INSR, CEACAM1, and IDE relative gene expressions and fasting plasma levels of albumin, total bilirubin, AST, and ALT of dogs

Dog no.	INSR	CEACAM1	IDE	Albumin (g/dL)*	Total bilirubin (mg/dL)†	AST (IU/L)‡	ALT (IU/L)§
1	0.32	0.45	0.49	3.3	0.2	21	31
2	1.77	0.21	0.24	3.6	0.2	20	30
3	0.03	1.39	3.05	3.1	0.1	15	17
4	4.54	0.45	1.68	2.5	0.3	23	56
5	3.31	0.40	0.46	3.4	0.2	22	26
6	5.87	0.61	1.10	3.3	0.2	34	40
7	4.14	3.49	0.22	3.2	0.2	33	36
8	2.35	0.92	0.32	3.2	0.2	28	27
9	3.45	0.21	0.19	3.3	0.2	28	41
10	3.33	2.01	2.16	2.7	0.1	20	24
11	5.2	2.46	5.17	3.3	0.1	33	52
12	7.72	1.08	8.58	3.4	0.1	23	41

INSR expression relative to 18s rRNA, CEACAM1 expression relative to 18s rRNA, and IDE expression relative to 18s rRNA, fasting plasma albumin, total bilirubin, AST, and ALT levels. ALT, alanine aminotransferase; AST, aspartate aminotransferase; IU, international unit. *Reference range 2.7–4.4 g/dL. †Reference range 0.1–0.3 mg/dL. ‡Reference range 15–66 IU/L. §Reference range 12–118 IU/L.

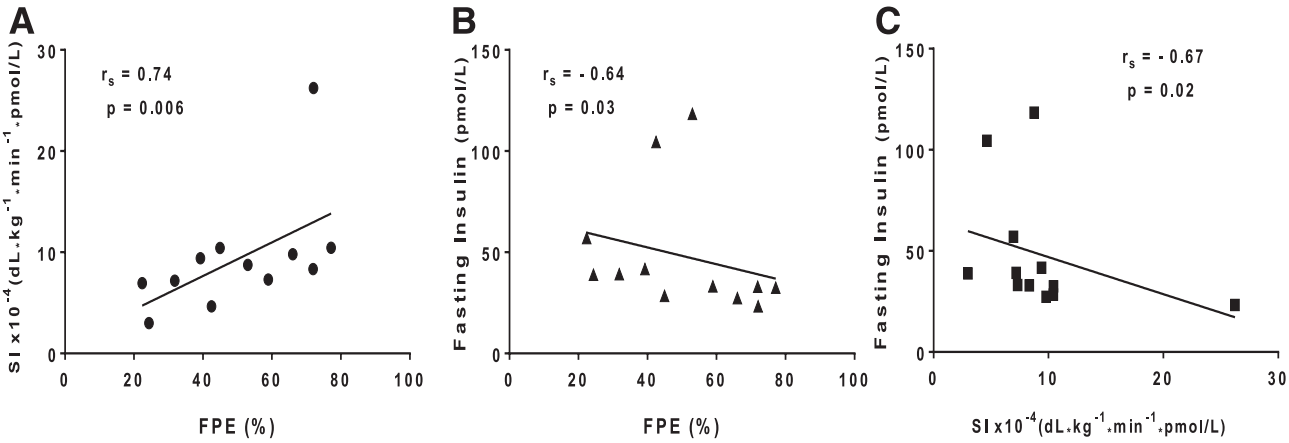

Figure 2—*A*: Correlation between FPE and SI. *B*: Correlation between FPE and fasting insulin. *C*: Correlation between SI and fasting insulin.

binding affinities of the alternatively spliced isoforms of the INSR IR-A and IR-B (23). IR-A binds to insulin with a higher affinity (about twice that of IR-B); however, in the liver, IR-A has a lower expression level than IR-B (23). Although the functional significance of the differences in binding affinities and expression levels of the isoforms is not completely clear, IR-A is reported to bind to CEACAM1 to mediate hepatic insulin extraction (15). In addition, HGO is both directly and indirectly controlled by insulin (11,24). Indirectly, secreted insulin allowed into systemic circulation after FPE regulation changes signals elsewhere in the body (e.g., central nervous system, adipose tissue lipolysis, glucagon secretion), which subsequently inhibit HGO (25,26). Thus, if the indirect control is dominant, there could be a dissociation between FPE and HGO/SiHGO. The strong correlation between FPE and SiP (r_s = 0.80; P = 0.002) (Fig. 3*A*) and the dissociation between FPE and HGO/SiHGO together support the dominance of indirect control of HGO by insulin. In addition, the lack of a significant correlation between basal HGO and relative expression of basal hepatic INSR does not indicate a dominant role for insulin's direct effect on HGO.

The metabolic clearance of insulin is reported to be the most formidable determinant of SI, much more so than fasting insulin, in overnight-fasted dogs (12). The strong correlation between FPE and SI (r_s = 0.74; P = 0.006) compared with fasting insulin and SI (r_s = −0.67; P = 0.02) reinforces the important role of insulin clearance in whole-body SI. Furthermore, the high variability of FPE (mean ± SD 50.4 ± 19.1%) (Table 1) seems to be reflected in the SI ($9.4 \pm 5.8 \times 10^4$ dL · kg^{-1} · min^{-1} · [pmol/L]$^{-1}$) and fasting insulin (48.1 ± 30.9 pmol/L).

Wide Interindividual Variation in FPE

The wide distribution of FPE in a cohort of normal animals is notable. The expansive intrapopulation differences, as evidenced in the FPE (Fig. 6), could be a result of genetic factors (27). Genetic association studies identified chromosomal regions linked to SI and insulin clearance (28). From studies in Mexican Americans, Goodarzi et al. (29) reported that metabolic clearance of insulin is highly heritable, and a follow-up study identified 18 associated single nucleotide polymorphisms (30). Such heritable allelic genes with varying degrees of penetrance possibly underscore the high variability in insulin clearance. FPE also seems to be a highly regulated process. After nutrient ingestion (31,32) and during marginal changes in body weight, even within normal BMI (17), FPE undergoes

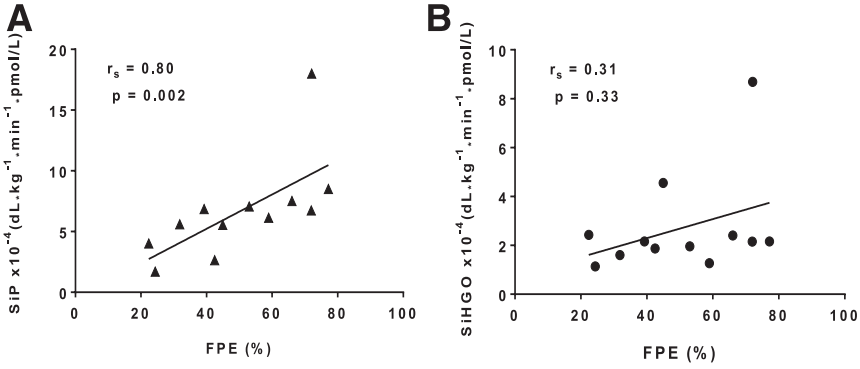

Figure 3—*A*: Correlation between FPE and SiP. *B*: Correlation between FPE and SiHGO.

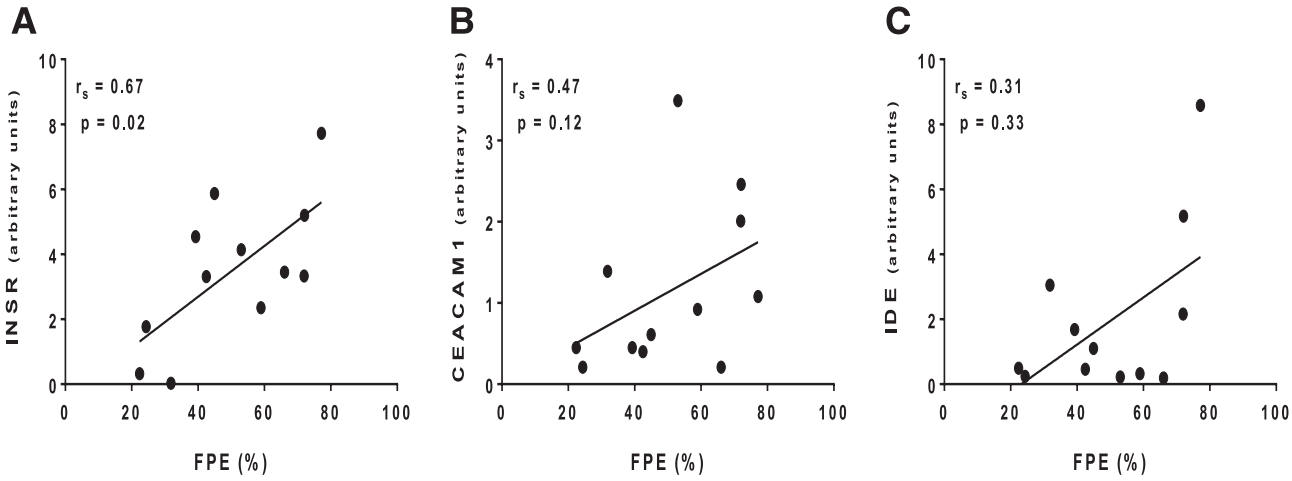

Figure 4—*A*: Correlation between FPE and INSR. *B*: Correlation between FPE and CEACAM1. *C*: Correlation between FPE and IDE.

dynamic changes to accommodate the insulin needs of the body, an indication of a tightly controlled mechanism. Perhaps low FPE signifies dysregulation of insulin clearance, which actuates hyperinsulinemia and insulin resistance.

CEACAM1 is involved in the internalization of the bound insulin on the hepatocytes (4,15), and in the endosome, IDE initiates the catabolism of insulin (10). The absence of a significant correlation among IDE, CEACAM1, and FPE (Fig. 4) in this study, despite their previously reported roles in hepatic insulin metabolism, might be a result of the lack of statistical power. IDE, although the principal catabolic enzyme of insulin (10), also is upregulated by insulin (33). Perhaps the increased expression levels of the gene during the hours-long insulin infusion of the PPII protocol might be significantly correlated with FPE and not with the basal expression assayed in this study. The significant association between FPE estimated through hyperinsulinemic conditions and the relative expression of INSR at basal (Fig. 4*A*) indicates the critical role of the INSRs in hepatic insulin extraction. Unlike insulin's control of HGO where the receptors are dispensable, hepatic insulin extraction requires the INSRs (24,34).

The wide variation of FPE recorded in this study also has been reported in other studies that used different methods. From direct sampling of the portal vein, hepatic artery, and hepatic vein with plasma flow rate measurements, Kaden et al. (35) reported a basal range of −20 to 90% (mean ± SD 40 ± 11%) in anesthetized dogs, and in conscious dogs, Jaspan and Polonsky (36) noted a sample mean of 31% (SD 11). In humans, using C-peptide deconvolution–derived insulin secretion and plasma insulin kinetics, Polonsky et al. (37) reported a mean of 53% at basal (SD 14) in 14 healthy weight individuals, whereas Meier et al. (38) reported a mean of 78% (SD 10) in 5. C-peptide is cosecreted with insulin, but unlike insulin, it is not significantly extracted by the liver; thus, the ratio of C-peptide to insulin is used as an index of FPE (39). Various clinical studies with hepatic vein sampling and peripherally infused insulin measured 40–85% single-pass splanchnic insulin extraction (39–41). Splanchnic insulin extraction encompasses insulin uptake by hepatic and

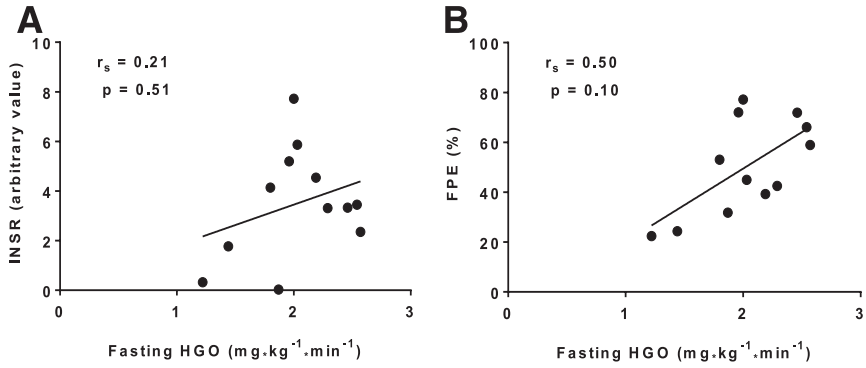

Figure 5—*A*: Correlation between fasting HGO and INSR. *B*: Correlation between fasting HGO and FPE.

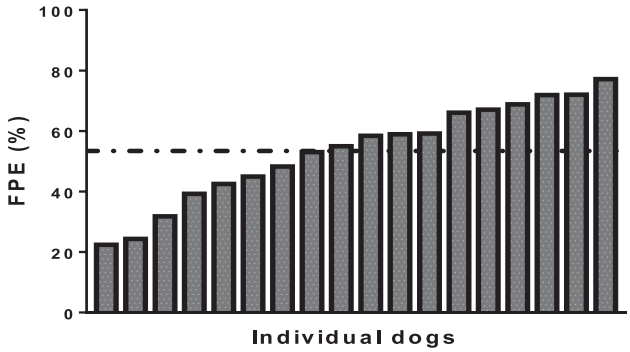

Figure 6—Distribution of FPE of 18 dogs. The mean of 53.4% is indicated by the dashed line (SD 19.5). Previously published baseline data of six dogs (3) were included to increase the sample size in the assessment of the FPE distribution.

extrahepatic visceral tissues, but the liver is the primary catabolic organ of the hormone (42).

Methods of Measuring FPE

The distinct advantage of the PPII method is that sampling is done only from a peripheral vein and not from deep-seated vessels (portal vein, hepatic artery, and hepatic vein), which are difficult to sample from. The unique challenge to the optimal representative sampling of portal blood because of streaming, the pulsatility of β-cell discharge, and sampling error (43–45) has resulted in disparate negative extraction values in some studies (35,45). Streaming as a result of the low velocity of portal blood could result in inadequate mixing of blood from the various vessels emptied into the portal vein (46–48). Sampling error (43–45) occurs as a result of the difficulty in drawing blood from these deep-seated vessels, which possibly confounds the measurement of hepatic extraction. Furthermore, the blood flow rate through a catheterized hepatic vein is altered compared with the other hepatic veins (49). Thus, estimating hepatic extraction fraction from one catheterized hepatic vein might not entirely reflect the composite liver uptake of insulin.

C-peptide–based methods of measuring FPE assume negligible C-peptide extraction by the liver. However, 3 of the 13 dogs used to accredit the insignificant average C-peptide uptake by the liver had appreciable hepatic C-peptide extraction values of 21–35% and another had a negative extraction percentage (45). Thus, in approximately one-quarter of the studied dogs (45), the C-peptide/insulin ratio could not be used as an accurate surrogate for FPE.

Nevertheless, PPII is limited by reliance on constant infusion rates and steady-state plasma insulin to calculate the FPE. Some studies reported that the pulsatile secretion of insulin determines its fractional hepatic extraction (38,50). In addition, PPII assumes that the plasma flow rate is the same for both experiments. Although the current sample mean of 53% is consistent with historical averages from endogenous pulsatile secretion (45), future studies will analyze the correlation between FPE from PPII and that of direct sampling from the hepatic artery and portal and hepatic veins. We also plan to conduct a longitudinal study to ascertain whether animals with low FPE develop insulin resistance and its attendant diseases relatively quickly compared with those with high or average FPE when subjected to a high-fat diet challenge. A limitation to this study is the inability to assess the two isoforms of the INSR and analyze how they relate with HGO and FPE.

Conclusions

By using a direct method of estimating FPE, the PPII, we found significant correlations among FPE, fasting insulin, and SI. Perhaps FPE regulates not only plasma insulin but also SI. In addition, we found a wide interindividual variation of FPE in normal healthy dogs, suggesting differential regulation of FPE. Poorly regulated low FPE might be a risk for hyperinsulinemia and insulin resistance.

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