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Fluid compartmental shifts with efficacious pioglitazone therapy in overweight monkeys: implications for peroxisome proliferator-activated receptor–γ agonist use in prediabetes

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Abstract

Pioglitazone is prescribed to improve insulin sensitivity in type 2 diabetes mellitus patients and has been discussed as a therapy for metabolic syndrome. Pioglitazone and other thiazolidinediones are associated with fluid retention and edema that may exacerbate existing or developing congestive heart failure, which is often present in these patients. Using a nonhuman primate model, our aims were to evaluate (1) whether fluid shifts were detectable in normoglycemic monkeys, (2) which fluid compartment changed, and (3) whether fluid retention was dose dependent. Seventeen adult male cynomolgus macaques (*Macaca fascicularis*) were studied in a Latin square design such that all animals received 0, 1, 2, and 5 mg/kg pioglitazone for 6 weeks with 2 weeks of washout between dosing intervals. Doses approximated human exposures achieved with 30, 45, and 60 mg. At the end of each period, animals were weighed and underwent dual-absorption x-ray absorption scanning for body composition measurements. Fluid volumes were quantitated by Evans blue dilution for plasma volume, equilibration of sodium bromide for extracellular water, and deuterated water for total body water. Significant ($P < .05$) effects were seen with expansion of PV at both the 2- and 5-mg/kg doses, along with reduced plasma sodium at 5 mg/kg; however, surrogate end points used to indicate fluid retention (body weight, hematocrit, total protein, and albumin) did not change significantly. Significant trends toward increases in interstitial fluid and extracellular water with increasing dose were apparent. Pioglitazone effectively improved metabolic status by significantly decreasing fasting glucose and triglycerides and increasing adiponectin. We conclude that thiazolidinedione-related plasma volume expansion occurs in nondiabetic primates and that fluid retention is detectable when compartments are directly measured.

1. Introduction

Type 2 diabetes mellitus (T2DM) and its complications incur significant social and economic burdens; and it has been shown that optimal control of blood glucose has lasting benefits and significantly modifies risk factors for cardiovascular disease [1], the most common cause of death in these patients [2]. It is generally accepted that the first stage of T2DM—sometimes referred to as a *prediabetic* state—involves peripheral (eg, muscle) insulin resistance and a compensatory hyperinsulinemia as the endocrine pancreas attempts to reestablish glucose homeostasis. Thiazolidinediones (TZDs) are peroxisome proliferator-activated receptor (PPAR) agonists, and drugs of this class improve insulin sensitivity and may preserve β-cell function when used in newly diagnosed T2DM patients [3]. Thiazolidinediones may be able to also reverse the prediabetic state and provide effective primary prevention for T2DM in insulin-resistant individuals [4]. Pioglitazone, an oral antidiabetic agent of the TZD class currently prescribed to improve insulin sensitivity in T2DM patients, has also been shown to decrease mortality in this population [5].

Clinical efficacy of TZDs is often accompanied by undesirable fluid accumulation that can result in weight gain and clinical edema. The fluid volume expansion seen in patients implies ongoing renal sodium and water retention. Peroxisome proliferator-activated receptor–γ receptors are present in the kidney (mesangial, collecting duct, pelvic urothelium, and renal vascular cells) [6,7], and activation of

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receptors in the distal nephron causes sodium retention [8,9]. Preclinical data from PPARγ collecting duct knockout mice indicate significant blunting, but not complete elimination, of TZD-associated volume expansion and that spironolactone is a more effective therapy than other diuretics for TZD-induced fluid retention after rosiglitazone [10], suggesting that renal mechanisms play a significant role in clinically relevant fluid retention [11].

In addition to direct effects on the kidney, PPARγ agonists have been shown to decrease blood pressure and peripheral vascular resistance in both human and animal studies [12]. The blood pressure and vascular resistance effects of TZDs may facilitate volume expansion under normal circumstances; however, the decreased vasorelaxation and vessel compliance common in hyperglycemic, hypertensive diabetic patients may lessen the ability to accommodate [13], such that TZD-associated volume expansion is more likely to present as peripheral edema and exacerbate existing or developing congestive heart failure, which is often present [12,14]. In patients with T2DM without evidence of cardiovascular dysfunction who are prescribed TZD monotherapy, volume expansion with fluid accumulation is seen in up to 5% of cases [14].

With trends toward earlier intervention in insulin resistance/prediabetes, evaluation of TZD safety and efficacy in healthier populations becomes relevant. Safety concerns regarding TZDs have supported current prescribing recommendations that indicate TZDs be used as adjunctive therapy to metformin and lifestyle modification or where severe dyslipidemias are present [15]. By daily dosing of pioglitazone in overweight, normoglycemic, but hyperinsulinemic monkeys, our aims in the current study were to determine (1) whether fluid shifts were detectable, (2) which fluid compartment had detectable changes, and (3) whether changes in fluid retention were dose-dependent. Furthermore, we evaluated efficacy end points to ascertain whether adverse fluid retention is induced at therapeutic dose levels. In addition, surrogate markers of efficacy were measured and compared with the dose-response for fluid volume expansion.

2. Methods

2.1. Experimental protocol

All experimental procedures involving animals during this study were carried out in accordance with *The Principles of Laboratory Animal Care* (National Institutes of Health publication no. 85-23, revised 1985) and in compliance with state and federal laws, standards of the US Department of Health and Human Services, and the instructions laid out by the Institutional Animal Care and Use Committee of Wake Forest University Health Sciences.

Seventeen mature obese male cynomolgus macaques (*Macaca fascicularis*) were included in the study. Monkeys were acclimated to and remained on a high-fat diet for 2 years after arrival at Wake Forest University Primate Center. At study start, they had gained on average 10% body weight (BW) on this diet and had more than doubled their fasting insulin concentrations (9.2 ± 1.2 vs 20.1 ± 2.6 μIU/L), with prediet values consistent with prior reports for this species [16]. Macronutrient content approximated a Western diet with fat accounting for 40.5%, protein 19.5%, and carbohydrates 40% of metabolizable energy. Monkeys were randomized to a 4-period Latin square design such that each monkey received placebo and 1-, 2-, and 5-mg/kg doses of pioglitazone (Actos; Takeda Chemical Industries, Lincolnshire, IL) as a single daily oral dose for 6 weeks. Dose levels were chosen based on limited pharmacokinetic information available in primates to approximate concentrations achieved with human doses of 30, 45, and 60 mg daily [17]. Two weeks were allowed between dosing periods for the drug effects to wash out (elimination $t_{1/2}$ approximating 5 hours in monkeys [18]). All clinical procedures described henceforth and blood sampling via femoral venipuncture were performed in animals fasted for 18 hours and sedated with ketamine hydrochloride (Ketaset; Fort Dodge, Fort Dodge, IA; 10-15 mg/kg IM).

2.2. Circulating blood parameters

At the end of each 6-week dosing interval, pioglitazone concentrations were measured 18 hours postdosing by quantitating the free base by liquid chromatography–mass spectrometry; and complete hematology and serum biochemistry panels were performed (GlaxoSmithKline, Research Triangle Park, NC). Glycosylation of hemoglobin chain A_{1c} was analyzed by borate affinity high-performance liquid chromatography (Primus PDQ, Kansas City, MO) to estimate longer-term glycemic control at 6 weeks of dosing. Adiponectin (Adpn) was quantitated by enzyme-linked immunosorbent assay supplied by Linco Research (St Charles, MO; expected coefficient of variation, <10%); and insulin, by enzyme-linked immunosorbent assay supplied by Mercodia (Winston-Salem, NC). Body weight, total plasma protein (TP), albumin (Alb), and hematocrit (Hct) were assessed as surrogate end points for changes in fluid status. Fasting glucose (Glu), hemoglobin A_{1c}, insulin, triglyceride (TG), free fatty acids (FFA), and Adpn were assessed as surrogate end points for the therapeutic effectiveness of pioglitazone. Homeostasis model assessment for insulin resistance (HOMA-IR) was calculated as the Glu (in millimoles per liter) * insulin (in micro–international units per milliliter)/22.5.

2.3. Fluid compartment volume estimations

Body composition was determined by dual energy x-ray absorption (DEXA) (Norland XR-46 X-Ray Bone Densitometer; Cooper Surgical, Trumbull, CT) analysis at 4 weeks of each dosing period and quantitated for lean and fat mass. Accurate normalization of all fluid volume measures was achieved by expression of each end point as a percentage of

lean body mass (LBM) [19] and corrected for the density of water at normal body temperature (0.99336 kg/L). Doses of agents administered were calculated by dose solution concentration and recording of the dose volume administered by syringe weight pre- and postinjection.

Plasma volume (PV), extracellular water (ECW), and total body water (TBW) were measured concurrently at the end of the 6-week dosing period. Intracellular water (ICW) was calculated as the difference between TBW and ECW. Evans blue dye (EB; Sigma-Aldrich, St Louis, MO), indicating Alb space (PV), was measured in plasma after administration of 2 mg/kg intravenously of a 4 mg/mL EB solution prepared immediately before injection. At preinjection and 5 minutes postinjection of EB, samples were collected; and plasma was analyzed for EB concentration, calculated from a standard curve generated by EB serial dilution in EB-free monkey plasma [8,20]. Repeatability was determined before experiment start in the study population and shown to have average interassay difference of 3% (data not shown) that is similar to previous reports [20].

Extracellular water was calculated from sodium bromide (NaBr) equilibration in plasma. Sodium bromide (Alfa Aesar, Ward Hill, MA) was dissolved in sterile water to a final concentration of 38.63 mg/mL and administered intravenously at a dose of 0.15 mL/kg. Plasma samples were collected pre- and 2-hours post–NaBr injection [21] and, along with the NaBr dosing solution, were measured for bromide concentrations by Metabolic Solutions (Nashua, NH). Extracellular water was determined from the corrected bromide space (CBS) using the following formula:

$$\mathrm{CBS} = ([\mathrm{Br}-]\ \mathrm{dose}/[\mathrm{Br}-]\ \mathrm{plasma}) * 0.90 * 0.95 * 0.94$$

where 0.90 is the correction for nonextracellular distribution, 0.95 is the Donnan equilibration factor, and 0.94 is the proportion of water in plasma. Repeatability for ECW was 6% in our study population.

Total body water was measured by deuterium oxide (D20) equilibration, and measurement of plasma deuterium (Metabolic Solutions) was by mass spectrometry as previously described [22]. Deuterium oxide was dosed intravenously at 0.142 mL/kg, and plasma was collected pre- and 2-hours postinjection. Total body water was determined using the following formula:

$$\mathrm{TBW} = [\mathrm{D20\ dose}/(\mathrm{D20}_{2h} - \mathrm{D20}_{0h})]/1.04$$

where 1.04 is the correction factor for deuterium bound to amino acids or other nonexchangeable sites [21]. Samples were measured in triplicate, with coefficients of variation typically less than 1%. Interstitial fluid volume (ISF) was calculated from the difference of ECW and PV.

2.4. Statistical analysis

All data in the text, tables, and figures are expressed as arithmetic mean values ± standard error of the mean (SEM), with P values for the main effects of treatment (dose of pioglitazone) unless otherwise indicated. Statistical analyses were performed using SAS versions 8.0.2 and 9.1.3 SP2 (SAS Institute, Cary, NC). Analysis initially included estimates of first-order carryover effects that were found to be insignificant and thus dropped from final models. Model factors included were subject, treatment (pioglitazone dose), and period for which main effects were estimated by mixed linear modeling. Log transformation of variables was performed when normality assumptions were not met (TBW, ECW, PV, ISF, BW, FFA, HOMA-IR). Pearson correlation between continuous variables was calculated. Trend analysis was performed using the dose code and outcome variables in Spearman rank-order correlations with α for all statistical comparisons set at .05.

3. Results

Plasma concentrations of pioglitazone from 18-hour postdose samples indicated successful administration of drug resulting in concentrations measured within the same range reported for humans [23]. At this time point, average plasma concentrations (SEM, n = 8-9 per group) were less than the limit of quantification for placebo and 411 (79), 621 (113), and 1007 (168) μg/L for 1-, 2-, and 5-mg/kg doses, respectively.

3.1. Fluid compartment volume results

Significant PV expansion was seen with the 2- and 5-mg/kg dose levels (Fig. 1). Volumes were 15% to 20% larger with these higher doses of pioglitazone treatment as compared with placebo. This increase in PV is also reflected in values for ECW, which showed a significant trend toward

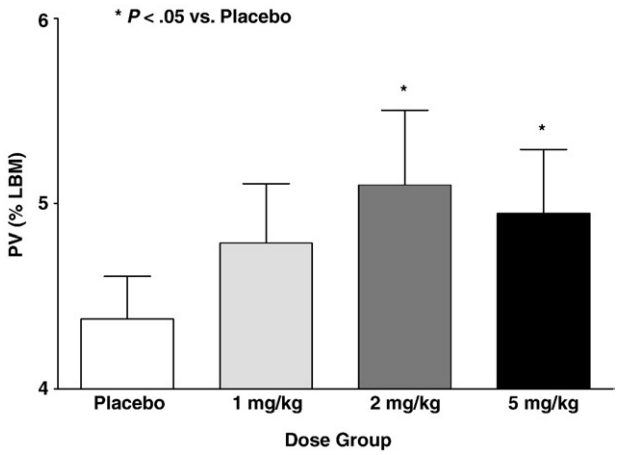

Fig. 1. Plasma volume estimates from EB dilution in monkeys (N = 17) dosed with pioglitazone for 6 weeks. Volumes calculated were corrected for individual LBM as measured by DEXA scanning. Plasma volumes show dose-dependent increases with significance ($P < .05$) achieved at the 2- and 5-mg/kg levels as compared with placebo.

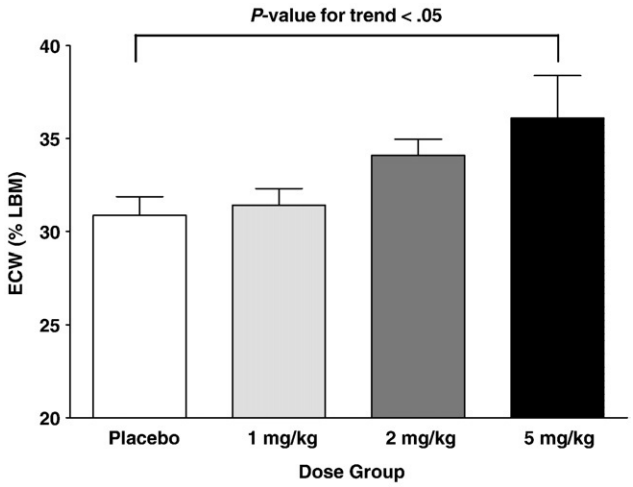

Fig. 2. Total ECW estimates from NaBr equilibration in monkeys (N = 17) dosed with pioglitazone for 6 weeks. Volumes calculated were corrected for individual LBM as measured by DEXA scanning. We observed a significant trend ($P < .05$) for increasing volumes of ECW with greater pioglitazone dosage.

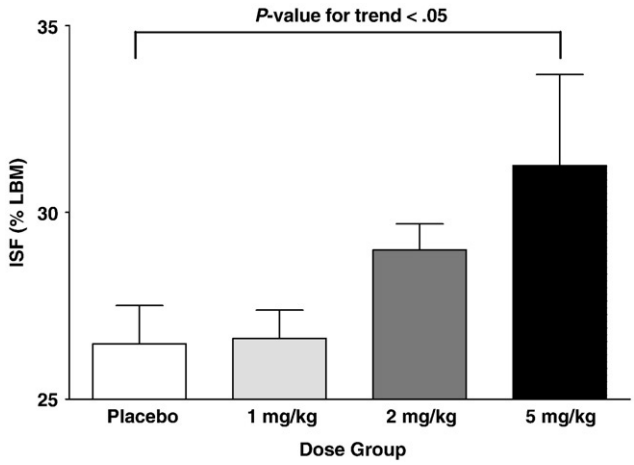

Fig. 3. Interstitial fluid volume, calculated from the difference between PV and ECW in monkeys (N = 17), after treatment with different doses of pioglitazone for 6 weeks. Volumes calculated were corrected for individual LBM as measured by DEXA scanning. We observed a significant trend ($P< .05$) for increasing volumes of ISF with greater pioglitazone dosage.

increases in the 2- and 5-mg/kg groups (Fig. 2). As expected, LBM and ICW were unaffected by treatment (Table 1).

Total body water (Table 1) only showed a tendency for increases at the highest dose level with a 3% increase as compared with placebo. This compartment has the greatest actual volume by accounting for all compartments; and thus, detection of treatment related differences has been, as expected, the most difficult [24].

The interstitial space (Fig. 3) is the compartment most representative of clinically apparent edema, as fluid collects outside the vascular space. A statistically significant trend toward increases in ISF was seen with more than 6% and 11% increases in volume at 2- and 5-mg/kg doses, respectively.

3.2. Surrogate end points for volume expansion

None of the evaluated surrogate end points for PV expansion or generalized fluid retention (Hct, Alb, TP, BW; Table 2) attained significance in the expected direction between the placebo and treatment periods. Body weight did not change despite evidence of fluid retention. Nonsignificant reduction in BW was seen in all treatment groups; and in light of observed increases in fluid volume, this likely indicates nonsignificant losses of fat mass during treatment (Table 1). It is worthy of note that both Hct and plasma Alb concentrations trended toward dose-dependent reductions consistent with hemodilution after PV expansion. An average BW change approximating 20 g would be expected from the PV expansion seen in these primates, which is too small a change to discern from measurement error in LBM by DEXA. Plasma sodium concentrations decreased with increasing pioglitazone dose, with mean (SEM) concentrations being 144.1 (0.34), 143.3 (0.27), 143.4 (0.42), and 142.8 (0.70) mEq/L after placebo and 1, 2, and 5 mg/kg. The reduction at the 5-mg/kg dose level was significant ($P = .01$). Potassium concentrations did not change significantly (data not shown).

3.3. Metabolic results

Indication of therapeutic efficacy was seen even in this study population of normoglycemic monkeys (Table 3). Fasting blood glucose was reduced at both 2 and 5 mg/kg, and plasma TG concentrations decreased at all dose levels. Adiponectin was significantly increased in a dose-dependent manner. Insulin concentrations did not change with treatment; but

Table 1
Fluid compartment and body mass measurements

	TBW (L)	TBW (%)	ICW (L)	ICW (%)	ECW (L)	ISF (L)	PV (L)	LBM (kg)	Fat mass (%)
Placebo	4.05 (0.14)	77.7 (0.78)	2.21 (0.09)	44.8 (1.03)	1.83 (0.15)	1.59 (0.10)	0.243 (0.02)	5.09 (0.19)	19.7 (2.75)
1 mg/kg	3.78 (0.14)	77.8 (1.65)	2.11 (0.07)	46.3 (1.7)	1.66 (0.08)	1.39 (0.07)	0.277 (0.02)	5.12 (0.22)	18.6 (2.53)
2 mg/kg	3.91 (0.16)	78.1 (1.20)	2.14 (0.12)	43.9 (1.59)	1.77 (0.07)	1.53 (0.06)	0.269 (0.02)	5.04 (0.18)	17.7 (2.48)
5 mg/kg	4.00 (0.28)	79.8 (0.89)	2.09 (0.18)	43.7 (2.07)	1.82 (0.13)	1.58 (0.14)	0.264 (0.03)	5.06 (0.22)	19.1 (2.61)

Values (mean ± SEM) for ECW, ICW, ISF, TBW, PV, fat (as percentage of total BW) mass, and LBM in obese hyperinsulinemic male monkeys (N = 17) dosed with pioglitazone for 6 weeks. Fluid measures are expressed as raw volumes (liters), and percentages were calculated from fluid weight (in kilograms) normalized to LBM (in kilograms) and expressed as percentage (%).

Table 2
Surrogate end points of fluid volume expansion

	BW (kg)	Hct (%)	TP (g/dL)	Alb (g/dL)
Placebo	6.85 (0.30)	38.4 (0.45)	7.88 (0.11)	4.06 (0.06)
1 mg/kg	6.80 (0.27)	37.4 (0.69)	7.77 (0.11)	3.99 (0.08)
2 mg/kg	6.70 (0.24)	37.9 (0.59)	7.82 (0.13)	3.86 (0.08)
5 mg/kg	6.84 (0.29)	37.5 (1.1)	7.91 (0.14)	3.88 (0.07)

All values (mean ± SEM) were measured in obese hyperinsulinemic male monkeys (N = 17) dosed with pioglitazone for 6 weeks. Body weight, Hct, TP, and Alb concentrations were evaluated; and although nonsignificant trends in Hct and Alb indicate hemodilution, no surrogate was sensitive enough to detect PV expansion.

FFA demonstrated a significant trend with decreases of 12.5%, 13.7%, and 30% as compared with placebo after 1, 2, and 5 mg/kg pioglitazone treatment for 6 weeks.

Because of the study duration, significant time effects were noted for all end points, which may have hampered the ability to detect statistically significant differences relating to dose. This finding is important, as the robustness of in vivo measures of TZD-induced volume expansion were assessed. Although a larger study population may have improved statistical comparisons, the simple estimation of PV was the most precise and accurate indicator of TZD-induced volume expansion.

4. Discussion

This study is the first to comprehensively evaluate fluid volume compartments after treatment with different dose levels of a TZD in a relevant nonhuman primate model of prediabetes. The results indicate that PV expansion occurs within 6 weeks of pioglitazone therapy at blood levels equivalent to clinically prescribed 45- and 60-mg doses. The lowest dose evaluated (1 mg/kg) was associated with metabolic improvement without significant changes in PV. This dose approximated the concentrations achieved after daily oral dosing of 30 mg pioglitazone in humans; and thus, we provide rationale for it being a safe and efficacious regimen for prediabetic patients.

In a smaller but similar study of obese rhesus monkeys, 3 mg/kg pioglitazone improved insulin sensitivity and resulted in significant reduction in blood pressure [17], as has been demonstrated in insulin-resistant [25,26] but not healthy subjects [27]. Mechanisms for blood pressure reduction are not fully elucidated; but vasodilation may result from PPAR activation, as receptors are present in vascular tissues [28,29]. Thiazolidinediones may directly cause arterial vasodilation, as evidenced by reduced systemic vascular resistance observed in T2DM patients [25]; and nonsignificant small blood pressure decreases in insulin-resistant but nondiabetic, obese subjects [26]. The arterial vasodilation, and subsequent arterial underfilling, effects a blood volume shift to the venous compartment. This may contribute to fluid retention by stimulating renal resorption of sodium by activation of the pressure-natriuresis system. Furthermore, PPARγ deletion or mutation of the ligand-binding region leads to hypertension in rodents and people [11,30,31]. We are limited in not having blood pressure measures, but the lower plasma sodium and volume expansion seen in the primates after treatment suggest that similar decreases in blood pressure may be present.

As vasodilation leading to volume expansion is unlikely to result in the consistent fluid retention seen with TZD therapy, investigation has been largely focused at the renal level, with identification of the distal nephron and collecting duct as the primary site of increased sodium retention [32]. Peroxisome proliferator-activated receptor−γ is expressed in the kidney, with the greatest abundance in the collecting duct [6,7]. Specific deletion of the receptor in the collecting duct significantly attenuated TZD-induced volume expansion and transient sodium retention [11] and was associated with increases in aldosterone, indicating that PPARγ receptor plays a necessary role in maintaining normal salt balance [27,33]. A PPARγ-mediated mechanism is further supported by observation of sodium reabsorption in the distal nephron by non-TZD PPAR agonists [8]. Logically, amiloride and spironolactone, diuretic agents known to act at the distal tubule and collecting duct, have been shown to be effective in reducing TZD-induced fluid retention [10,33].

Vasodilation may account for residual fluid retention as discussed, and this in concert with increased vascular permeability may permit the generation of edema. Evidence for leakage of fluid from the vascular space has been reported

Table 3
Plasma concentrations of metabolic end points

	Glu (mg/dL)	Insulin (μIU/mL)	TG (mg/dL)	FFA (mEq/L)	Adpn (ng/mL)	HOMA IR
Placebo	61.7 (1.94)	20.1 (2.62)	44.2 (5.84)	0.80 (0.08)	22.7 (6.19)	2.87 (0.39)
1 mg/kg	63.6 (1.42)	21.2 (2.70)	39.7 (6.11)*	0.70 (0.07)	58.6 (13.7)*	3.10 (0.36)
2 mg/kg	60.4 (2.10)*	24.7 (4.16)	34 (4.48)*	0.69 (0.11)	74.6 (18.6)*	3.55 (0.58)
5 mg/kg	59.6 (1.80)*	24.4 (5.18)	38.2 (9.83)*	0.56 (0.05)‡	114 (21.7)*,†	3.70 (0.91)

All values (mean ± SEM) for metabolic end points indicating therapeutic efficacy of pioglitazone were measured in obese hyperinsulinemic male monkeys (N = 17) after 6 weeks of dosing. Fasting blood glucose, TG, and Adpn concentrations significantly improved with therapy; and nonesterified FFA concentrations and HOMA indices trended toward improvement.

* $P \leq .05$ as compared with placebo.

† $P \leq .05$ as compared with other dose groups.

‡ $P < .05$ for trend.

after rosiglitazone in vitro, with reversible dose-dependent increases in cell permeability observed in pulmonary cell culture after exposure to high concentrations [34]. On the other hand, no change in the fraction of tagged Alb leaving the vascular space was measured in a study of obese, insulin-resistant people after treatment for 12 weeks [26].

The vasodilatory action of TZDs may partly affect insulin sensitization, as increased perfusion of capillary beds allows greater glucose uptake in tissues such as muscle [35]. Insulin per se induces vascular dilatation and increases renal sodium resorption in the healthy individual, whereas T2DM people are resistant to this effect [35,36]. By restoring insulin sensitivity with TZD therapy, individuals with high circulating insulin concentrations or those on insulin therapy are at risk for augmented volume expansion. This has been suggested by significant association between insulin sensitivity estimates and edema formation [26]. Our study did not result in any correlations between fasting glucose and volumes measured from any fluid compartment, which may have been a function of the animal model having only moderately elevated insulin concentrations.

Clinical trials and animal studies have relied heavily on surrogate end points such as Hct when screening for TZD-related adverse events [17,24,37]. Our results indicate that an average 0.5% reduction in Hct is associated with significant PV expansion. This magnitude of change is consistent with criteria used in clinical trials to select patients prone to the development of fluid retention with TZD therapy [10] and was smaller than the Hct change seen in T2DM patients [24]. No significant changes in surrogate end points of fluid volumes were seen in rhesus monkeys administered pioglitazone doses similar to ours in cynomolgus monkeys [17]. Hemodilution is not consistently apparent with TZD use. For example, Hct changes were not seen after high-dose pioglitazone (45 mg) therapy for T2DM patients despite significantly increased TBW as measured by the same methodology used in this study [25], whereas Hct significantly decreased in similarly dosed T2DM people with no measurable change in TBW [24]. Hematocrit also did not change in healthy men with measurable sodium retention due to pioglitazone [27].

Plasma proteins are rarely used as an indicator of hemodilution, although our data, along with others [10,37], suggest that Alb has a similar capacity to Hct to reflect dose-related volume expansion. We observed an almost 20% increase in PV after adjustment for body size; however, in an approximately 7-kg monkey, this amounts to small changes in volume that are not great enough to significantly shift concentrations of surrogates measured per 100 mL of blood (Tables 1 and 2). Only plasma Alb concentrations changed in a dose-dependent manner, such that there was a 5% decrease at the highest pioglitazone dose suggesting hemodilution. We conclude that measurable PV expansion and hemodilution occur in healthy individuals. Edema, as indicated by the trend for ISF accumulation, occurred but would not be expected to become clinically apparent until ECW has increased by 30% or total weight is increased by 10% [38].

Clinically, the development of edema and weight gain is similar between the 2 currently licensed drugs, pioglitazone and rosiglitazone [14]. Body weight is commonly increased with TZD therapy, but is flawed as a surrogate because weight gain results from both fluid retention and adipogenesis and thus lacks specificity [24,39]. As in the other pioglitazone nonhuman primate study [17], we did not record reliable increases in BW or fat over the duration of the study. The absence of measurable weight gain during TZD administration has been seen in studies of rodents and both T2DM and healthy people where fluid retention has been directly measured [25,27,40]. From our results, the PV increase in an average human after pioglitazone would approximate 300 g, which is surprisingly close to the average weight gain seen in T2DM people that could not be attributed to adipogenesis [24]. Although Hct significantly decreased in that study, TBW was unchanged; and thus, hemodilution was ruled out. For this reason, the comparison of all surrogate end points with measures of all volume compartments within this study is advantageous. It allows us to conclude that, in a healthier population, PV is superior to surrogate end points in detecting fluid retention and is a technically simple and rapid test.

We conclude that, in the evaluation of the capacity of the PPARγ agonists (pioglitazone) to induce volume expansion or edema, nondiabetic primates are a valid model in which we were able to detect TZD-related fluid retention coupled with metabolic efficacy end points. Although we were able to show trends toward ECW and ISF accumulation, the most sensitive and significant change was in increases in PV. As this compartment is rarely measured clinically, incorrect conclusions about fluid retention in nondiabetic people have been suggested [25,27]. Volume expansion is indeed seen with TZD treatment in a dose-dependent manner, with the healthy animal able to prevent the accumulation of clinically apparent edema with the expansion of the vascular space. Thus, as a clinically relevant indicator of volume expansion, measurement of PV would be more useful to identify increased drug-related fluid retention in the absence of clinical edema or congestive symptoms. Clinicians need to be alerted to these clinically silent hemodynamic changes and should be aware of their potential impact on the cardiovascular system in prediabetic and T2DM patients.

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