Quantification of the Effects of Troglitazone on Insulin Sensitivity and β-Cell Function in Watanabe Heritable Hyperlipidemic Rabbits: A Minimal Model Analysis

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Troglitazone is a newly developed antidiabetic drug that has been shown to improve insulin resistance and hyperinsulinemia both in diabetic animal models and in patients with non–insulin-dependent diabetes mellitus. The Watanabe heritable hyperlipidemic (WHHL) rabbit, an animal model of familial hypercholesterolemia, is characterized by hyperinsulinemia, which reflects insulin resistance. In this study to determine the effects of troglitazone on glucose and insulin metabolism in WHHL rabbits, we quantified the rate of glucose utilization (glucose tolerance index [Kg]), sensitivity of first-phase posthepatic insulin secretion to glucose (Φ_1), sensitivity of second-phase posthepatic insulin secretion to glucose (Φ_2), insulin sensitivity to glucose disposal ([S_i] inversely related to insulin resistance), insulin-independent glucose disposal (glucose effectiveness [S_g]), and rate of insulin clearance (K_i) by incorporating our previously reported two-compartment model of a glucose/insulin system with the glucose disappearance model of Bergman. Galvin insulin sensitivity (GIS) was also computed for comparison with Bergman S_i. When troglitazone was administered as a food admixture (24 mg/d per animal) for 6 months, it did not significantly affect β-cell function as measured by Φ_2, glucose tolerance as measured by K_g, or S_g, but increased both S_i and K_i and reduced Φ_1, leading to a decreased plasma insulin response during the intravenous glucose tolerance test (IVGTT). S_i was strongly and significantly correlated with GIS. These data indicate that in WHHL rabbits, troglitazone improves insulin sensitivity and posthepatic insulin clearance without affecting β-cell function or glucose tolerance.

OUR PREVIOUS EXPERIMENTAL study[1] with Watanabe heritable hyperlipidemic (WHHL) rabbits demonstrated an association between insulin resistance and atherosclerosis. WHHL rabbits, an animal model of familial hypercholesterolemia, are characterized by hyperinsulinemia both in the fasting state and during the intravenous glucose tolerance test (IVGTT) and by relatively normal glucose tolerance.[1] Another study[2] showed that angiotensin-converting enzyme inhibitor, which has been shown to have antiatherogenic effects in WHHL rabbits,[3] reduced both the exaggerated insulin response during the IVGTT and insulin resistance in WHHL rabbits. Since insulin resistance and hyperinsulinemia play a crucial role in the pathogenesis of atherosclerosis, treatments that improve the action of insulin could be beneficial.

Troglitazone, also known as CS-045, has been shown to improve insulin resistance and hyperinsulinemia in diabetic animal models,[4] patients with non–insulin-dependent diabetes mellitus,[5,6] and obese subjects without non–insulin-dependent diabetes mellitus.[7] It appears to work by enhancing insulin action[4,5,8] without stimulating β-cell insulin secretion.[4,9] This agent has also been effective in preventing nonhyperglycemic insulin resistance and hypertriglyceridemia in fructose-induced insulin-resistant rats.[10] Therefore, since hypertension markedly stimulates atherosclerosis in WHHL rabbits[11] and since troglitazone is effective in reducing blood pressure in diabetic hypertensives[12] and in obese Zucker rats,[13] we were encouraged to use troglitazone to treat insulin resistance and hyperinsulinemia in WHHL rabbits.

Hyperinsulinemia could result from resistance to insulin-mediated glucose disposal[14] and impairment of the immediate insulin response to glucose.[15] Understanding the etiology of hyperinsulinemia requires techniques for measuring both pancreatic responsiveness to glucose and insulin sensitivity. In this study, we used a new model-based noninvasive approach to quantify both pancreatic responsiveness and insulin sensitivity in the intact organism.[16,17] This method, the minimal model technique, uses computer modeling to analyze plasma glucose and insulin dynamics during the IVGTT with frequent sampling. Our previously described two-compartment mathematical model of glucose/insulin metabolism[2] was used to obtain characteristic parameters for the rate of glucose utilization (glucose tolerance index [K_g]), sensitivity of first-phase posthepatic insulin delivery to glucose (Φ_1), sensitivity of second-phase posthepatic insulin delivery to glucose (Φ_2), and rate of insulin clearance (K_i). Posthepatic insulin secretory responsiveness to glucose (both Φ_1 and Φ_2) were defined according to Toffolo et al.[17] Bergman's model of glucose disappearance, in which parameters are determined using an analytical method[2] instead of a numerical method,[16] was used to obtain estimates of insulin sensitivity to glucose disposal ([S_i] inversely related to insulin resistance) and insulin-independent glucose disposal ([S_g] glucose effectiveness).[16]

In the present study to examine the effects of troglitazone on glucose and insulin metabolism in WHHL rabbits, we modeled the dynamics of glucose and insulin during the IVGTT to measure glucose tolerance, glucose effectiveness, insulin sensitivity, first- and second-phase posthepatic insulin secretion, and insulin removal before and after 6 months of treatment with troglitazone.

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MATERIALS AND METHODS

Animals

Female homozygous WHHL rabbits (N = 12; age, 11 months; body weight, 2.07 to 3.04 kg) kindly provided by Dr M. Shiomi (Kobe University, Kobe, Japan) were housed individually in an environmentally controlled room (Animal Center, Fukuoka University School of Medicine, Fukuoka, Japan) with a 12-hour light/dark cycle (light 7 AM to 7 PM). The rabbits had free access to water and were fed standard rabbit chow (RC-4; Oriental Yeast, Tokyo, Japan) at 80 g/d per animal. Rabbits were acclimated for 2 weeks before troglitazone treatment was started. The project was approved by the Ethics Committee of Fukuoka University.

Troglitazone Treatment

Six rabbits were randomly selected to be treated with troglitazone, and the rest (n = 6) were used as controls. Troglitazone, a generous gift from Sankyo (Tokyo, Japan) was administered at 10:30 AM as a food admixture (0.03%) that was freshly mixed every month and stored at 4°C. The treated rabbits were fed this food admixture at 80 g/d per animal, while the control rabbits continued to eat standard rabbit chow RC-4 at 80 g/d per animal. Since WHHL rabbits completely ingest this amount of food in 2 to 6 hours, each rabbit consumed the same amount of troglitazone, ie, 24 mg per animal. This dosage (24 mg/2.5 kg body weight per animal) is comparable to a non-obese dosage in humans (400 mg/60 kg body weight) and twice an obese dosage (400 mg/100 kg body weight). This treatment lasted for 6 months.

IVGTT

An IVGTT was performed before treatment and at 6 months of treatment, as reported previously.[1,2]

Glucose and Insulin Measurements

Plasma glucose was assayed by the glucose oxidase procedure (Glucoroder-MK II; Analytical Instruments, Tokyo, Japan) immediately after the IVGTT, and plasma for insulin determination was stored at −80°C until assayed. Immunoreactive insulin (IRI) in plasma was determined by radioimmunoassay (Insulin Kit; Eiken Chemical, Tokyo, Japan). The procedure for the human Insulin Kit was modified by reducing the amount of antibody and increasing the amount of ^{125}I-labeled antigen[18] to make the most precise portion of the standard curve coincide with the range of rabbit insulin levels (2.5 to 80 μU/mL) without increasing the counting error. The samples were measured in duplicate for plasma glucose concentrations (coefficient of variation, <5%) and in triplicate for insulin concentrations (coefficient of variation, <10%).

Drug Levels

At 6 months, 3 mL fasting blood was drawn, and the plasma was stored at −80°C until measurement. The drug level was measured using high-performance liquid chromatography (Sankyo).[19]

Modeling Analysis

The modeling of IVGTT glucose and insulin concentrations and the derivation of parameters of insulin sensitivity and dynamics were performed by incorporating our previously described two-compartment model of the glucose/insulin system[2] (model 1) and the glucose disappearance model of Bergman et al[16] (model 2). In model 1, first- and second-phase posthepatic insulin secretory responses were defined according to Toffolo et al.[17] In model 2, the differential equations that describe the disappearance of glucose and the appearance of insulin action were integrated using an analytical method[20] for convenience and ease of application for parameter identification in this study. Model-derived parameters are defined below, and the model itself and a mathematical derivation of the parameters are described in the Appendix.

Model 1. The slope analysis method described by Hlad and Elrik[21] and the equation we described previously were used to derive the following parameters: (1) Kg, the rate constant of glucose utilization, a measure of glucose tolerance (units, per hour); (2) G_b, the glucose concentration in the basal (steady) state, which is assumed to be reached at 120 minutes into the IVGTT (units, milligrams per deciliter); (3) Φ_1, the integral concentration of insulin delivered during the first phase of insulin secretion relative to the maximum increase in glucose concentration above the basal (steady) level (units, microunits per milliliter per hour per milligram per deciliter); (4) Φ_2, a constant relating the rate of increase in the insulin concentration in the second phase of insulin secretion to the glucose concentration above the basal (steady) level (units, microunits per milliliter per hour per milligram per deciliter); (5) K_i, the fractional clearance rate of insulin ($\log_e 2$/insulin half-life; units, per hour); (6) I_b, the insulin concentration in the basal (steady) state, which is assumed to be reached at 120 minutes into the IVGTT (units, microunits per milliliter); and (7) Galvin insulin sensitivity (GIS), defined as glucose disappearance (K_g) per unit insulin increase (area under the IVGTT curve) above the basal level from 0 to 40 minutes after glucose loading.[22]

Model 2. This model predicts the plasma glucose concentration from the insulin concentration. The derived parameters are (1) S_i, Bergman insulin sensitivity, which is the fractional rate constant for insulin-dependent glucose disposal, a measure of the sensitivity of glucose disposal to insulin (units, per hour per microunit per milliliter); and (2) S_g, glucose effectiveness, which is the fractional rate constant for net insulin-independent glucose disposal, a measure of the ability of glucose to enhance its own disappearance (units, per hour).

The parameters described were identified using a nonlinear regression analysis included in the SAS software package (Version 6, Statistical Analysis System; SAS Institute, Cary, NC) and by incorporating the two models already presented. The model procedure,[23] which includes dynamic modeling capabilities and methods for parameter estimation in nonlinear systems of equations, was used to perform nonlinear regression analysis. The ordinary least-squares method was used for parameter estimation. The Marquardt iterative method was used for minimization. The initial values were obtained by a grid search: the parameters are unique if the initial values are chosen properly.[2] Monte Carlo simulation of the model procedure was used to generate error boundaries of parameters estimated using the model. One set of parameters was obtained for each rabbit.

Statistical Analysis

Statistical analysis was performed using the SAS software package (Version 6, Statistical Analysis System). The distribution of variables was examined by the univariate procedure,[24] which tests the normality of variables and gives the median and interquartile ranges of variables. Due to the small sample size in this study, it was difficult to determine the correct distribution of variables. Thus, both parametric and nonparametric methods were used for data analysis. Nonparametric analyses were performed by analyzing the ranks of the variables using parameteric methods. Ranks of variables were produced by the rank procedure.[24] Associations between variables were measured by the Pearson product-moment correlation and Spearman rank correlation using the correlation (CORR) procedure.[24] Between-group differences at 0 and 6 months were compared by a one-way ANOVA using the general linear models (GLM) procedure[25] and/or by a nonparametric Wilcoxon-Mann-Whitney test using the nonparametric one-way (NPAR1WAY) procedure.[25] The one-way and two-way multivariate repeated-measures analysis was used to test within-subject changes for each group and within-subject by between-subject interaction effects.[25] Plasma levels of glucose and insulin and model parameters are presented as the median

and quartile range, and blood concentrations of the drug are presented as the mean ± SE. The significance level was considered to be 5%.

RESULTS

Plasma Troglitazone Concentrations

Table 1 shows the concentrations of troglitazone and its metabolites detected in overnight-fasting plasma drawn from troglitazone-treated rabbits after 6 months of treatment. These results indicate that a once-per-day administration of troglitazone in the morning is sufficient to maintain blood concentrations of troglitazone for over 20 hours. The overnight-fasting drug levels in rabbits (4.17 ± 0.20 μg/mL) were 3.7 times the maximum levels (1.13 ± 0.14 μg/mL) in humans when the drug was administered orally twice per day in the fasting state at a dose of 400 mg,[26] and were 5.5 times the maximum levels (0.76 μg/mL) in humans when the drug was administered once per day.[19] The different effective drug levels in animals and humans are apparently due to the medication method, since in humans the effective drug levels were higher for administration twice per day versus once per day even when the same dose was administered (1.13 ± 0.14 *v* 0.76 ± 0.12 μg/mL)[19,26] and were higher when the drug was administered after dinner than in the fasting state (0.70 ± 0.12 *v* 0.50 ± 0.08 μg/mL, at a dosage of 200 mg once per day).[19]

Analysis of IVGTT

The goal of our analytical approach is to quantitatively account for the effects of troglitazone on glucose and insulin metabolism in hyperinsulinemic WHHL rabbits. To achieve this, we used the minimal model to describe the IVGTT data from each rabbit.

Model fitting ability. Figure 1 shows (for two rabbits after 6 months of treatment) the ability of the insulin kinetics model to describe insulin kinetics given the glucose concentrations (Appendix, model 1, Eq 2) and the ability of the glucose disappearance model to describe glucose kinetics given the insulin concentrations (Appendix, model 2, Eq 3 and Eq 4). Figure 1A shows plots of insulin concentrations versus time during the IVGTT. Figure 1B shows plots of glucose concentrations versus time. The model-predicted time courses of glucose and insulin showed a good fit to the experimental data for glucose and insulin, respectively.

Results of the IVGTT. Figure 2 shows the time course of plasma glucose and IRI concentrations during the IVGTT for control and troglitazone-treated rabbits at 0 months and 6 months of treatment. Each set of glucose points is associated with the model-predicted glucose profiles, and each set of insulin points is associated with the model-predicted insulin profiles. The magnitude of glucose curves did not change markedly in control or troglitazone-treated rabbits with treatment (6 months). The magnitude of the insulin curve in control rabbits also did not change significantly with treatment (6 months). On the other hand, the magnitude of the insulin curve

Table 1. Fasting Plasma Concentrations of Troglitazone and Its Metabolites After 6 Months of Treatment

	Control	Troglitazone-Treated
Troglitazone (μg/mL)	—	4.17 ± 0.20
Metabolites (μg/mL)		
Sulfate	—	12.40 ± 1.38
Quinone	—	1.08 ± 0.04

NOTE. Data are the mean ± SE.

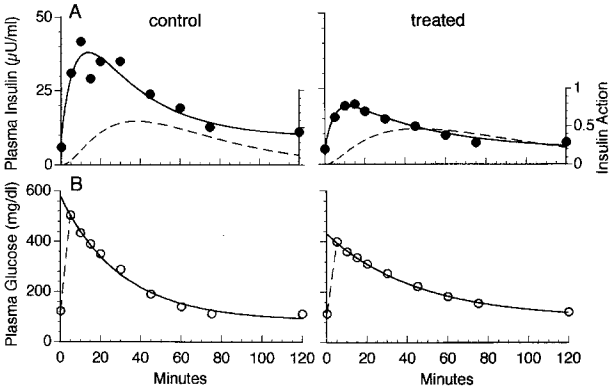

Fig 1. Ability of the minimal models to describe glucose and insulin kinetics during the IVGTT. Left and right panels show typical control and troglitazone-treated rabbits at 6 months, respectively. (——) Model-predicted time courses of plasma insulin and glucose; (– – –) time courses of insulin action (X in model 2) in upper panels, interpolated lines between data points in lower panels.

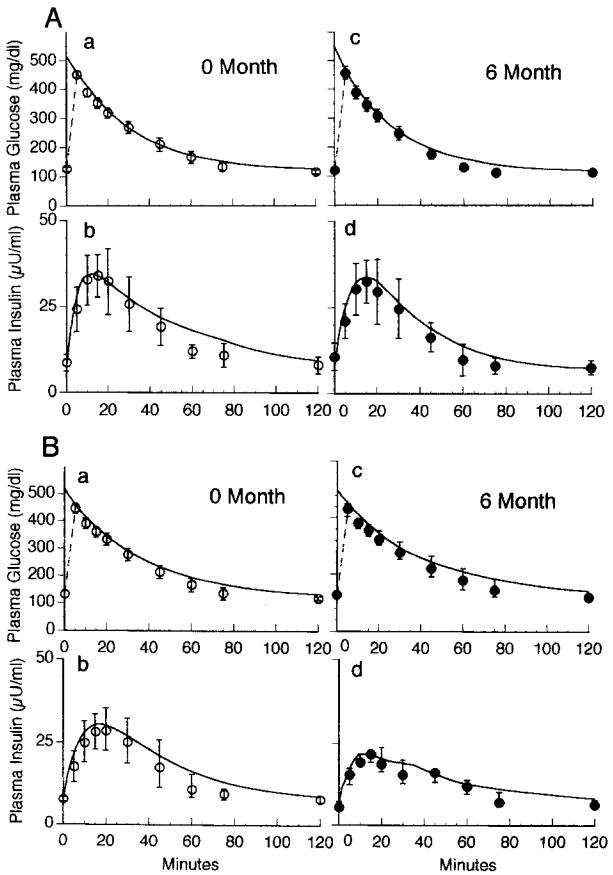

Fig 2. Time courses of plasma glucose and IRI during the IVGTT in control (A) and troglitazone-treated (B) rabbits at 0 months and 6 months. Experimental data points are presented as medians with quartile ranges. (——) Model-fitted trajectories; (– – –) interpolated between experimental data points.

in troglitazone-treated rabbits changed markedly: insulin responses at 6 months were smaller than those before treatment and smaller than those in control rabbits both before and after 6 months of treatment.

Characteristic Metabolic Parameters

Characteristic metabolic parameters for control and troglitazone-treated rabbits are listed in Tables 2 and 3, respectively. Table 2 and Table 3 show individual data for all control and treated rabbits and list the mean ± SD with significant changes (within-subject effects) during the study for troglitazone-treated and control rabbits, as well as within-subject by between-subject interaction effects (Table 3) as assessed by a multivariate repeated-measures ANOVA.[25] No significant changes in the model parameters were observed for control rabbits. For troglitazone-treated rabbits, no significant changes were observed for basal (steady-state) glucose concentrations (G_b), glucose utilization rate (K_g), insulin-independent glucose disposal (S_g), or second-phase posthepatic insulin responsiveness to glucose (Φ_2). A tendency for decreased basal insulin concentrations (I_b) and significant decreases in the peak insulin response (second-phase) to glucose (I_{peak}) and first-phase posthepatic insulin responsiveness to glucose (Φ_1) were observed in treated rabbits (Table 3). The rate of insulin clearance (K_i) and insulin sensitivity to glucose disposal (S_i) were significantly increased in treated rabbits. Within-subject by between-subject interaction effects assessed by a two-way multivariate repeated-measures ANOVA were observed for Φ_1, I_{peak}, K_i, and S_i, indicating that compared with values in control rabbits, troglitazone significantly decreased the insulin response to glucose as measured by I_{peak} and first-phase posthepatic insulin secretion as measured by Φ_1, and significantly increased insulin sensitivity as measured by S_i and the insulin clearance rate as measured by K_i. The increased insulin clearance rate constant K_i in troglitazone-treated rabbits gave a shorter half-life of insulin after 6 months of treatment (5.0 *v* 5.6 minutes, $P < .05$).

Table 4 shows the fractional standard deviation of parameter estimates for model 1 and model 2 to demonstrate the validity of the modeling analysis method. All of the parameters of model 1 (Appendix, Eq 1 and Eq 2) and model 2 (Appendix, Eq 3 and Eq 4) were estimated within 50% of the parameter value. The fractional standard deviations of the parameter estimates of model 2, which was used to calculate S_i and S_g, were extremely small (within 5%) compared with those reported by others (between 50% and 80%).[16] Considering that we used a 10-sample minimal model analysis, it should be difficult to achieve this level of accuracy. However, since we incorporated model 1 and model 2, not only the experimental values but also the predicted values for glucose and insulin concentrations from model 1 are input into model 2 for parameter estimation. These predicted values are calculated at 1-minute intervals. Therefore, the data points used for parameter estimation in model 2 are 10 + 120. Thus, it is reasonable that we should obtain highly accurate parameter estimates.

Comparison of Bergman Insulin Sensitivity to GIS

We computed GIS (Tables 2 and 3; Appendix, model 1) for comparison to Bergman insulin sensitivity (Appendix, model

Table 2. Characteristic Parameters for Control Rabbits at 0 and 6 Months

Rabbit No.	BW (kg)	G_b (mg/dL)	I_b (μU/mL)	I_{peak} (μU/mL)	K_g (h^{-1})	Φ_1 ($\times 10^{-2}$ μU/mL · h^{-1})/(mg/dL)	Φ_2 (μU/mL · h^{-1})/(mg/dL)	K_i (h^{-1})	GIS (h^{-1})/(μU/mL)	S_g (h^{-1})	S_i ($\times 10^{-2}$ h^{-1})/(μU/mL)
1											
0 mo	2.6	103	6.5	31.9	1.9	3.7	1.00	6.2	0.090	0.73	2.6
6 mo	2.9	101	5.7	25.1	2.4	5.0	0.67	5.0	0.145	0.43	3.4
2											
0 mo	2.4	118	5.9	17.0	1.9	0.8	1.05	12.8	0.174	1.05	4.5
6 mo	2.6	113	6.5	14.1	2.2	0.6	1.03	14.6	0.227	1.00	5.2
3											
0 mo	2.7	111	7.8	26.4	1.6	1.2	1.29	11.1	0.092	1.39	2.8
6 mo	2.8	113	9.2	19.6	2.2	2.0	0.83	7.7	0.150	0.79	3.6
4											
0 mo	2.6	117	7.5	15.8	1.2	0.7	0.88	11.9	0.092	1.07	3.7
6 mo	2.8	120	7.7	16.3	2.6	1.9	0.76	7.8	0.223	0.68	4.5
5											
0 mo	2.5	120	12.7	12.6	2.1	1.0	0.93	10.7	0.169	1.14	4.1
6 mo	2.7	112	11.0	18.0	2.0	1.5	0.74	8.1	0.132	0.98	3.5
6											
0 mo	2.6	124	7.5	12.9	2.2	3.9	0.49	4.8	0.201	0.65	5.1
6 mo	2.7	123	5.9	10.3	2.1	1.5	0.45	6.7	0.245	0.83	6.3
Mean 0 mo	2.6	116	8.0	19.4	1.8	1.9	0.94	9.6	0.136	1.01	3.8
SD	0.1	7	2.4	7.9	0.4	1.5	0.26	3.3	0.050	0.27	1.0
Mean 6 mo	2.8*	114	7.7	17.2	2.3	2.1	0.75	8.3	0.187	0.79	4.4
SD	0.1	8	2.1	5.0	0.2	1.5	0.19	3.3	0.050	0.21	1.2

NOTE. Calculations and abbreviations for model parameters are described in the Appendix.

Abbreviation: BW, body weight.

*$P < .05$ *v* 0 months (within-subject effect).

Table 3. Characteristic Parameters for Troglitazone-Treated Rabbits at 0 and 6 Months

Rabbit No.	BW (kg)	G_b (mg/dL)	I_b (μU/mL)	I_{peak} (μU/mL)	K_g (h^{-1})	Φ_1 ($\times 10^{-2}$ μU/mL · h^{-1})/(mg/dL)	Φ_2 (μU/mL · h^{-1})/(mg/dL)	K_i (h^{-1})	GIS (h^{-1})/(μU/mL)	S_g (h^{-1})	S_i ($\times 10^{-2}$ h^{-1})/(μU/mL)
7											
0 mo	2.2	118	6.6	8.7	1.6	0.9	0.40	7.5	0.187	1.51	6.1
8											
0 mo	2.4	110	9.5	25.7	1.6	3.3	0.81	5.8	0.083	1.11	2.7
6 mo	2.7	120	6.4	13.6	1.5	1.1	0.59	8.3	0.135	1.57	4.6
9											
0 mo	2.4	119	9.3	11.9	1.3	1.2	0.55	7.4	0.108	1.86	4.2
6 mo	2.6	128	8.3	5.3	1.6	0.3	0.33	10.9	0.094	1.32	6.3
10											
0 mo	2.1	127	7.2	16.3	1.7	1.7	0.74	7.5	0.136	1.15	4.1
6 mo	2.3	121	7.3	7.2	1.3	0.5	0.72	12.8	0.174	1.16	6.3
11											
0 mo	2.4	123	8.0	10.5	1.9	1.4	0.57	7.5	0.200	1.03	5.4
6 mo	2.6	131	5.7	8.3	2.0	1.4	0.42	6.5	0.267	0.91	7.2
12											
0 mo	2.4	116	7.7	15.4	2.0	3.6	0.53	4.9	0.169	0.71	4.4
6 mo	2.4	114	5.3	12.6	1.5	1.1	0.55	7.9	0.157	1.46	5.2
Mean 0 mo	2.3	119	8.3	16.0§	1.7	2.2§	0.64	6.6§	0.139	1.17	4.2§
SD	0.1	7	1.0	5.9	0.3	1.1	0.13	1.2	0.047	0.42	1.0
Mean 6 mo	2.5*	123	6.6†	9.4‡	1.6	0.9*	0.52	9.3*	0.165	1.28	5.9‡
SD	0.2	7	1.2	3.6	0.3	0.5	0.15	2.5	0.064	0.26	1.0

NOTE. Parameters for rabbit no. 7 at 6 months were not calculated because sampling for some time points during the IVGTT failed.
*$P < .05$, †$P < .1$, ‡$P < .01$: *v* 0 months (within-subject effect).
§$P < .05$, within-subject by between-subject interaction effect.

2). When the ranks of insulin sensitivity derived from model 2 (Bergman insulin sensitivity) and model 1 (GIS) were compared, a highly significant correlation was observed between the combined data for troglitazone-treated and control rabbits using the two methods ($r_s = .68$, $P = .0004$; Fig 3).

In summary, the main effect of troglitazone was that it improved insulin sensitivity (S_i) and insulin clearance (K_i) without affecting second-phase β-cell responsiveness (ϕ_2) in WHHL rabbits.

DISCUSSION

Our previous experimental studies[1,2] with WHHL rabbits, an established animal model of familial hypercholesterolemia, have demonstrated that insulin resistance is associated with atherosclerosis. We have also quantitatively characterized the insulin-glucose response in WHHL rabbits using the minimal model approach.[2] Parameters such as the rate constant of glucose utilization, the rate constant of insulin secretion (second-phase), and the rate constant of insulin clearance have been determined. However, quantitative measurements of insulin-independent glucose disposal (glucose effectiveness), insulin sensitivity, and first-phase β-cell insulin responsiveness have not been made. Since hyperinsulinemia could result not only from insulin resistance[14] but also from an impaired immediate insulin response (first-phase β-cell responsiveness),[15] the quantitative measurement of insulin sensitivity and first- and second-phase β-cell insulin secretion is important for understanding the etiology of hyperinsulinemia. It is also meaningful to divide total glucose uptake into insulin-dependent and insulin-

Table 4. Accuracy of Parameter Estimates

Fractional Standard Deviation of Parameter Estimates (%)									
Model 1						Model 2			
G_b	ΔG	K_g	I_b	Φ_2	K_i	P_1	P_2	P_3	P_4
12	3	9	34	20	24	1.0	0.5	1.3	0.6

NOTE. Results are the mean for 12 rabbits.

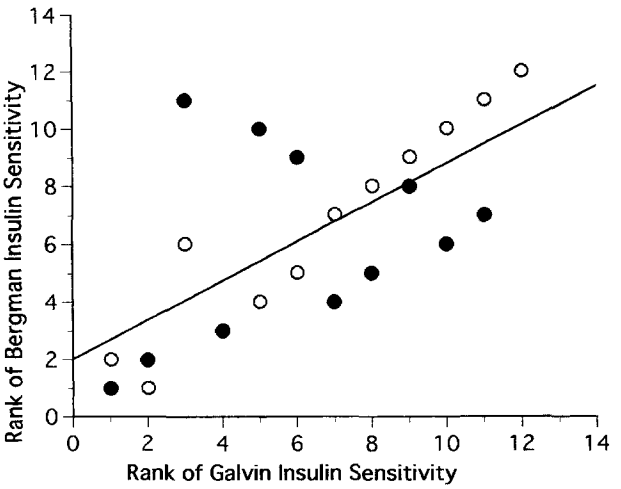

Fig 3. Comparison of Bergman insulin sensitivity obtained from model 2 with GIS obtained from model 1 ($r_s = .68$, $P = .0004$). (○) Control rabbits; (●) troglitazone-treated rabbits. All data are ranks obtained from the original data for troglitazone-treated and control rabbits.

independent factors,[27] ie, to distinguish insulin sensitivity from glucose effectiveness, which is glucose uptake independent of a dynamic change in insulin concentration.[16,28,29]

In the present study, we incorporated our previously reported two-compartment model of the glucose-insulin system[2] and the glucose disappearance model of Bergman et al[16] to simultaneously assess glucose utilization (K_g), first- and second-phase β-cell responsiveness (Φ_1 and Φ_2), insulin clearance rate (K_i), insulin sensitivity (S_i), and glucose effectiveness (S_g) in WHHL rabbits. We also computed GIS for comparison to Bergman insulin sensitivity (S_i). In this study, insulin sensitivity is expressed as microunits per milliliter per hour as opposed to per minute as originally used by Bergman et al.[16] This caused the calculated value for insulin sensitivity (S_i) to be approximately 100-fold higher for rabbits than that reported for humans.[30] Since we used a similar unit in our previous report,[2] we continued to use this expression for convenience in comparing parameters other than insulin sensitivity, eg, K_g, K_i, Φ_2, with our previous report.

Troglitazone is a new agent for which the mechanism of action involves improving insulin resistance in Zucker rats[4] and humans.[5,6] In this study, we examined the effects of troglitazone on hyperinsulinemia and insulin resistance in WHHL rabbits.

Twelve WHHL rabbits were used in this study. Although using more animals would have been preferable to achieve more reliable statistical results, homogeneous WHHL rabbits are bred only at Kobe University in Japan and are difficult to produce. Dr Shiomi of Kobe University was kind enough to give us 12 homogeneous WHHL rabbits, but could not provide any more.

Godsland et al[31] suggested that changes in Φ_1 and Φ_2 may only represent apparent changes in insulin secretion because the model of insulin dynamics describes posthepatic insulin delivery rather than pancreatic insulin secretion, since first-phase hepatic insulin uptake is not corrected.[17] It has also been suggested that first-phase insulin secretion responsiveness (Φ_1) is not a sensitive measure of islet function,[32] since this measurement depends not only on the amount of insulin released but also on the rate of insulin clearance by hepatic and peripheral mechanisms,[29] the prevailing insulin sensitivity,[31] and ambient glucose.[33] However, the usefulness of Φ_2 as an index of β-cell function has been validated in three studies that compared Φ_2 with pancreatic insulin secretion as assessed by the acute insulin response to arginine at three different clamped glucose concentrations,[34-36] and Φ_2 has therefore been used by Abel and Ledingham[37] as an index of β-cell function in hypertensive patients.

Iwamoto et al[5] reported that troglitazone (CS-045) does not stimulate insulin secretion, since the insulin response during an oral glucose tolerance test either decreased or did not change. Our results are consistent with these findings, and the modeling techniques we used indicated that troglitazone neither stimulated nor suppressed β-cell function in WHHL rabbits, since Φ_2 was not significantly affected by troglitazone treatment.

Troglitazone-treated rabbits showed a significant reduction in first-phase insulin responsiveness (Φ_1). Since Φ_1 represents posthepatic insulin delivery, a reduction in Φ_1 may result from an increase in hepatic uptake. However, since second-phase β-cell responsiveness to glucose (Φ_2) remained unchanged, this may not be the correct interpretation, because if hepatic insulin uptake was increased, an overall reduction in first- and second-phase β-cell responsiveness (Φ_1 and Φ_2) would be expected. Toffolo et al[17] have pointed out that first-phase insulin secretion depends on the rate of insulin clearance. The modeling technique in our study also indicated a significant increase in the rate of insulin clearance (K_i).

The most striking effects of troglitazone included reductions in the fasting insulin concentration (Fig 2B) and the insulin response to glucose injection (Table 3 and Fig 2B). Godsland et al[31] demonstrated that factors contributing to changes in the insulin response could be resolved as changes in insulin secretion resulting from changes in insulin sensitivity and changes in insulin clearance by applying a model of insulin dynamics. Our study, which also applied a modeling technique, confirms this notion and indicates that troglitazone increased both insulin sensitivity (S_i) and insulin clearance (K_i) (Table 3).

Our previous report[2] suggested that states of insulin resistance in WHHL rabbits are associated with a reduced rate of insulin removal. Our present data confirm this point: the changes in the model-derived parameters S_i and K_i indicate that troglitazone improves insulin sensitivity and insulin clearance. The modeling technique is not able to separate insulin-independent and insulin-dependent mechanisms of glucose disposal into hepatic and peripheral components.[38] However, since the liver is the principal site of insulin removal,[39] the increased rate of insulin clearance suggests that troglitazone at least improves hepatic insulin sensitivity. A recent report in streptozotocin-induced diabetic rats has demonstrated that troglitazone improves hepatic insulin resistance.[40] In addition, other agents that improve hepatic insulin sensitivity such as glyburide also increase hepatic insulin clearance.[41]

Anderson et al[42] indicated that GIS calculated from the frequently sampled IVGTT was strongly and consistently associated with insulin sensitivity computed from glucose clamped over a wide range of glucose tolerance. Therefore, we compared GIS[22] derived from model 1 with Bergman insulin sensitivity derived from model 2, which discriminates between insulin-dependent glucose disposal and insulin-independent glucose disposal.[16] The results show that Bergman insulin sensitivity is strongly and significantly correlated with GIS (Fig 3).

In summary, use of the minimal model approach to interpret changes in glucose and insulin concentrations under conditions of glucose loading was useful for quantifying the effects of troglitazone on insulin secretion and insulin sensitivity in WHHL rabbits. Troglitazone improved insulin sensitivity and insulin clearance and did not affect β-cell function in the WHHL rabbit. Although troglitazone has been reported to increase insulin sensitivity in other animal species[4] and humans,[5-7] this is the first study to show this effect of troglitazone in a hyperlipidemic model, WHHL rabbits. Since troglitazone also decreased blood pressure in diabetic hypertensives,[12] as well as in this study (data not shown), it may be useful for the insulin resistance syndrome. This is also the first study to evaluate the effects of troglitazone on glucose/insulin metabolism using 10-sample minimal models.

APPENDIX: DERIVATION OF GLUCOSE AND INSULIN PARAMETERS

Model 1: Two-Compartment Model of the Glucose/Insulin System[2]

The time course of the glucose concentration derived from a glucose kinetics equation[2] is expressed as the sum of an exponential and a constant,

$$G(t) = \Delta G \cdot e^{-K_g \cdot t} + G_b, \qquad \text{Eq 1}$$

where $G(t)$ is plasma glucose concentration at time t, ΔG is the maximum increase in glucose concentration above the basal level due to glucose injection, and G_b is glucose concentration in the basal (steady) state. This equation better describes the curve of the decreasing plasma glucose concentration in rabbits than the simple exponential equation introduced by Hamilton and Stein[43] that is used as a standard method for calculating K_g. Amatuzio et al[44] have also demonstrated in a human study that introduction of a term for fasting blood glucose concentration to the simple exponential equation results in an equation that better describes the data.

However, the same results for the specific rate constant K_g can be obtained using the slope-analysis method described by Hlad and Elrik[21] regardless of the equation used. In practice, Eq 1 was fitted to the experimentally determined glucose data to estimate the parameters of the glucose curve. The glucose utilization rate constant (K_g) equaled the slope of the $\log_e$ (predicted glucose concentration) versus time relationship after glucose injection.[21]

Using the derived parameters, the incremental area of glucose (IGA) under the IVGTT curve was calculated between 0 and 120 minutes:

$$IGA = \int_0^{120} (G(t) - G_b) \cdot dt = \frac{\Delta G}{Kg} \cdot (1 - e^{-K_g \cdot 120/60}).$$

The incremental response, as expressed in terms of the incremental area under the IVGTT curves, provides a measure of the IVGTT response unconfounded by changes in the basal (steady) level.

The time course of the insulin concentration derived from an insulin kinetics equation[2] is expressed as

$$I(t) = \frac{\Phi_2 \cdot \Delta G}{K_i - K_g} \cdot (e^{-K_g \cdot t} - e^{-K_i \cdot t}) + I_b, \qquad \text{Eq 2}$$

where $I(t)$ is plasma insulin concentration at time t, Φ_2 is second-phase β-cell responsiveness to glucose according to Toffolo et al,[17] K_i is the rate constant of insulin clearance, and I_b is insulin concentration in the basal (steady) state. In practice, Eq 2 was fitted to the experimentally determined insulin data to estimate the parameters of the insulin curve. Φ_1 is calculated according to Toffolo et al.[17]

The time at which the second-phase insulin secretory response reaches its peak insulin value is calculated from Eq 3 as

$$t_{max} = \frac{\ln\left(\frac{K_i}{K_g}\right)}{(K_i - K_g)}.$$

The peak value (I_{peak}) is calculated from Eq 2 with t_{max} instead of time t. Using the derived parameters, the incremental insulin area (IIA) under the IVGTT curve was calculated between 0 and 120 minutes:

$$IIA = \int_0^{120} (I(t) - I_b) \cdot dt = \frac{\Phi_2 \cdot \Delta G}{(K_i - K_g)} \cdot \left[\frac{1}{K_g} \cdot (1 - e^{-K_g \cdot 120/60}) - \frac{1}{K_i} \cdot (1 - e^{-K_i \cdot 120/60})\right].$$

IIA from 0 to 40 minutes after glucose loading was calculated as

$$IIA_{0\text{-}40} = \int_0^{40} (I(t) - I_b) \cdot dt = \frac{\Phi_2 \cdot \Delta G}{(K_i - K_g)} \cdot \left[\frac{1}{K_g} \cdot (1 - e^{-K_g \cdot 40/60}) - \frac{1}{K_i} \cdot (1 - e^{-K_i \cdot 40/60})\right].$$

Therefore, GIS[22] is $K_g/IIA_{0\text{-}40}$.

Model 2. Glucose Disappearance Model of Bergman et al[16,28,29]

This model of glucose dynamics is represented mathematically as[28,38]

$$\frac{dG(t)}{dt} = (P_1 - X) \cdot [G(t) - G_b] + P_4, \qquad \text{Eq 3}$$

where P_4 is net hepatic glucose balance in the basal state, X is insulin's effectiveness in promoting glucose uptake, and P_1 is a constant.

The suprabasal insulin action at time t[$X(t)$] depends on the incremental insulin response above the basal level. This dependency is quantified by considering suprabasal insulin as the result of the tendency (P_3) for suprabasal insulin to become active, balanced against the tendency (P_2) for suprabasal insulin action to dissipate. The rate of change of insulin action is mathematically represented as

$$\frac{dX(t)}{dt} = P_3 \cdot [I(t) - I_b] - P_2 \cdot X(t). \qquad \text{Eq 4}$$

By replacing Eq 2 into Eq 4 and integrating Eq 4 analytically using the Laplace transform,[20] insulin action at time t is

$$X(t) = \frac{P_3 \cdot \Phi_2 \cdot \Delta G}{K_i - K_g} \cdot \left[\frac{e^{-K_g \cdot t}}{P_2 - K_g} - \frac{e^{-K_i \cdot t}}{P_2 - K_i} - \left(\frac{1}{P_2 - K_g} - \frac{1}{P_2 - K_i} \right) \cdot e^{-P_2 \cdot t} \right]. \quad \text{Eq 5}$$

Eq 5 is replaced into Eq 3, and Eq 3 is fitted to the time course of plasma glucose predicted from Eq 1 to obtain estimates of P_1, P_2, and P_3.

Bergman's insulin sensitivity, S_i, is defined as[16]

$$S_i = \frac{P_2}{P_3}.$$

Insuline-independent glucose uptake, Sg, is defined as[16]

$$S_g = P1 - X_{\text{steady state}}.$$

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