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Original Research

Hepatic and Whole-Body Insulin Metabolism during Proestrus and Estrus in Mongrel Dogs

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Insulin resistance occurs during various stages of the estrus cycle in dogs. To quantify the effects of proestrus–estrus (PE) and determine whether PE affects liver insulin sensitivity, 11 female mongrel dogs were implanted with sampling and intraportal infusion catheters. Five of the dogs (PE group) entered proestrus after surgery; those remaining in anestrus were controls. The dogs were fasted overnight, [3-^{3}H]glucose and somatostatin were infused through peripheral veins, and glucagon was infused intraportally. Insulin was infused intraportally, with the rate adjusted to maintain arterial plasma glucose at basal levels (PE, 294 ± 25 µU/kg/min; control, 223 ± 21 µU/kg/min). Subsequently the insulin infusion rate was increased by 0.2 mU/kg/min for 120 min (P1) and then to 1.5 mU/kg/min for the last 120 min (P2); glucose was infused peripherally as needed to maintain euglycemia. Insulin concentrations did not differ between groups at any time; they increased 3 µU/mL over baseline during P1 and to 3 times baseline during P2. The glucose infusion rate in PE dogs during P2 was 63% of that in control dogs. Net hepatic glucose output and the endogenous glucose production rate declined 40% to 50% from baseline in both groups during P1; during P2, both groups exhibited a low rate of net hepatic glucose uptake with full suppression of endogenous glucose production. The glucose disappearance rate during P1 and P2 was 35% greater in control than PE dogs. Therefore, PE in canines is associated with loss of nonhepatic (primarily muscle) but not hepatic insulin sensitivity.

Abbreviations: NEFA, nonesterified fatty acids; P1, low-dose hyperinsulinemic clamp period (0–120 min); P2, high-dose hyperinsulinemic clamp period (120–240 min); PE, proestrus and estrus

Estrus and diestrus are associated with a loss of insulin sensitivity in both normal and diabetic dogs[2,8,31,33,35] Although muscle is known to be involved in this change in insulin sensitivity,[30] whether the liver is affected also is unclear. The liver is an especially important organ of glucoregulation, because it both releases glucose to and extracts it from the circulation, depending on glycemic and hormonal cues. However, to our knowledge the liver's response to insulin in the period surrounding estrus has not been examined nor has the degree of insulin resistance been quantified in depth.

Because dogs are an important model for humans in metabolic research[26,28] and a popular companion animal prone to developing diabetes, insulin resistance, and impaired glucose tolerance,[32] understanding metabolic consequences of the normal estrus cycle in dogs is important. In addition, the NIH and other research funding agencies currently require or strongly encourage the acquisition of data from both sexes in applicable preclinical studies,[6,15,34] thus highlighting the importance of elucidating the effects of the estrus cycle on the canine model.

In the current project, we sought to compare hepatic and peripheral insulin sensitivity and glucose metabolism in normal female dogs in PE and anestrus. These studies were accomplished by using a 2-step hyperinsulinemic euglycemic clamp after the insulin infusion rate that maintained basal glycemia (that is, reflecting near-basal insulin secretion) had been established. The first data-collection period involved an insulin infusion rate previously demonstrated to suppress endogenous glucose production by approximately 50% in normal female dogs in anestrus, and the second period used an infusion rate known to completely suppress endogenous glucose production and to increase whole-body glucose utilization by approximately 7-fold in normal dogs of both sexes.[5] We chose the hyperinsulinemic euglycemic clamp technique for these studies because it is frequently considered to be the 'gold standard' for assessment of insulin sensitivity in humans and animal models.[1,17,20,40,41] The use of 2 insulin-infusion rates allows the differentiation of hepatic and whole-body insulin sensitivity, given that the liver's response is evident at a much lower insulin delivery rate than that of peripheral insulin-sensitive tissues.[4,10,27]

Materials and Methods

Animals and surgical procedures. The protocol was approved by the Vanderbilt University IACUC, and all animals were housed in a facility accredited by AAALAC. Adult female mongrel dogs (n = 11; weight, 23.0 ± 0.4 kg) obtained from a USDA-licensed vendor were studied. All dogs consumed a diet of dry chow and canned food (Laboratory Canine Diet 5006, LabDiet, St Louis, MO; Pedigree Choice Cuts, Mars Petcare US, Franklin, TN; metabolizable energy, approximately 1650 to 1850 kcal/d; 31% protein, 26% fat, and 42% carbohydrate).

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Approximately 16 to 17 d prior to the experiment, the dogs underwent laparotomy and surgical insertion of splenic and jejunal vein infusion catheters for infusions into the hepatic portal circulation as well as the insertion of sampling catheters in the left femoral artery, hepatic portal vein, and left common hepatic vein. Ultrasonic flow probes (Transonic Systems, Ithaca, NY) were placed around the hepatic artery and portal vein. The incision was closed in 3 layers. All dogs received analgesics preoperatively (0.02 mg/kg buprenorphine) and for 48 h postoperatively (0.02 mg/kg buprenorphine and 0.1–0.2 mg/kg meloxicam). During the postoperative period, dogs were observed at least twice daily by research and veterinary staff. Criteria for use and the preparation for study were as previously described.[9] The control group consisted of 6 dogs in anestrus. Dogs in the proestrus–estrus group (PE; $n = 5$) were determined by vaginal cytology to have entered proestrus 6 ± 5 d (range 1 to 23) prior to study.

Clamp studies. The dogs were fasted for 18 h prior to study. On the morning of study, the catheters and flow probes were removed from their subcutaneous pockets under the use of local anesthesia. At –130 min, a basal arterial plasma glucose sample was obtained, and a primed (35 µCi), constant infusion (0.35 µCi/min) of [3-^{3}H]glucose (PerkinElmer, Waltham, MA) was initiated through a peripheral vein. Concurrently, a continuous infusion of somatostatin (0.8 µg/kg/min; Bachem, Torrance, CA) was begun through a different peripheral vein. In addition, infusions of human insulin (approximately 0.3 mU/kg/min; Eli Lilly, Indianapolis, IN) and glucagon (GlucaGen, Novo Nordisk, Denmark; 0.57 ng/kg/min) were delivered into the splenic and jejunal catheters at rates previously shown to yield near-basal hormone concentrations in overnight-fasted dogs for example.[9,12] The splenic and jejunal veins drain into the hepatic portal vein, the route by which insulin and glucagon reach the liver during normal secretion. Both hormones were replaced because somatostatin suppresses the pancreatic secretion of glucagon as well as insulin. Arterial plasma glucose concentrations were obtained every 5 min thereafter, and the insulin infusion rate was adjusted as necessary to maintain arterial plasma glucose concentrations at the basal level.

Table 1. Plasma insulin and glucagon concentrations and total hepatic blood flow

	Experimental period		
	Basal	P1	P2
Arterial insulin (µU/mL)			
control	7 ± 1	10 ± 1	28 ± 3
PE	6 ± 1	8 ± 1	29 ± 3
Arterial glucagon (ng/L)			
control	35 ± 3	35 ± 2	31 ± 3
PE	40 ± 5	37 ± 2	34 ± 2
Hepatic blood flow (mL/kg/min)			
control	25 ± 5	27 ± 5	26 ± 4
PE	23 ± 3	24 ± 3	24 ± 3

Data are mean ± SEM (control, $n = 6$; PE, $n = 5$) for 3 sampling times in each period. There were no significant differences between groups.

After stable insulin infusion rates were obtained at approximately –50 min, no further insulin adjustments were made during the equilibration and basal periods. The period between –30 and 0 min served as a basal sampling period. After the 0-min sample was obtained, the insulin infusion rate was increased by 0.2 mU/kg/min; this continued for 120 min (0 to 120 min; test period 1 [P1]). At 120 min, the insulin infusion rate in all dogs was increased to 1.5 mU/kg/min, and this second test period (P2) lasted from 120 to 240 min. During the basal period, P1, and P2, blood samples were collected from the artery and the portal and hepatic veins at designated intervals, by using techniques previously described.[9]

Analytical procedures. Analyses have been described in detail previously[9] and included plasma concentrations of glucose, insulin, glucagon, cortisol, nonesterified fatty acids (NEFA), and ^{3}H glucose and blood concentrations of lactate, alanine, glycerol, and β-hydroxybutyrate.

Calculations.

$$\text{Net hepatic substrate balance} = \text{LOAD}_{\text{out}} - \text{LOAD}_{\text{in}}$$

$$\text{LOAD}_{\text{out}} = \text{hepatic vein substrate concentration} \times \text{total hepatic blood or plasma flow as appropriate for the particular substrate, and}$$

$$\text{LOAD}_{\text{in}} = (\text{arterial substrate concentration} \times \text{arterial blood or plasma flow}) + (\text{portal vein substrate concentration} \times \text{portal vein blood or plasma flow.}$$

Nonhepatic glucose uptake was calculated as the glucose infusion rate minus the net hepatic glucose output, with the rate corrected for changes in the size of the glucose pool, as previously described.[7] Glucose turnover (rates of endogenous appearance and disappearance) was calculated according to the circulatory model described previously.[21,22]

Statistical comparisons were made using 2-way repeated-measures ANOVA with posthoc analysis by using the Tukey test or an unpaired t test; a P value of less than 0.05 was accepted as statistically significant. Systat software (San Jose, CA) was used for statistical analysis. All data in the text are expressed as mean ± SEM of the 3 sampling times during the last half hour of each period.

Results

Insulin infusion rates, plasma hormone concentrations, and hepatic blood flow. The insulin infusion rates required to mimic

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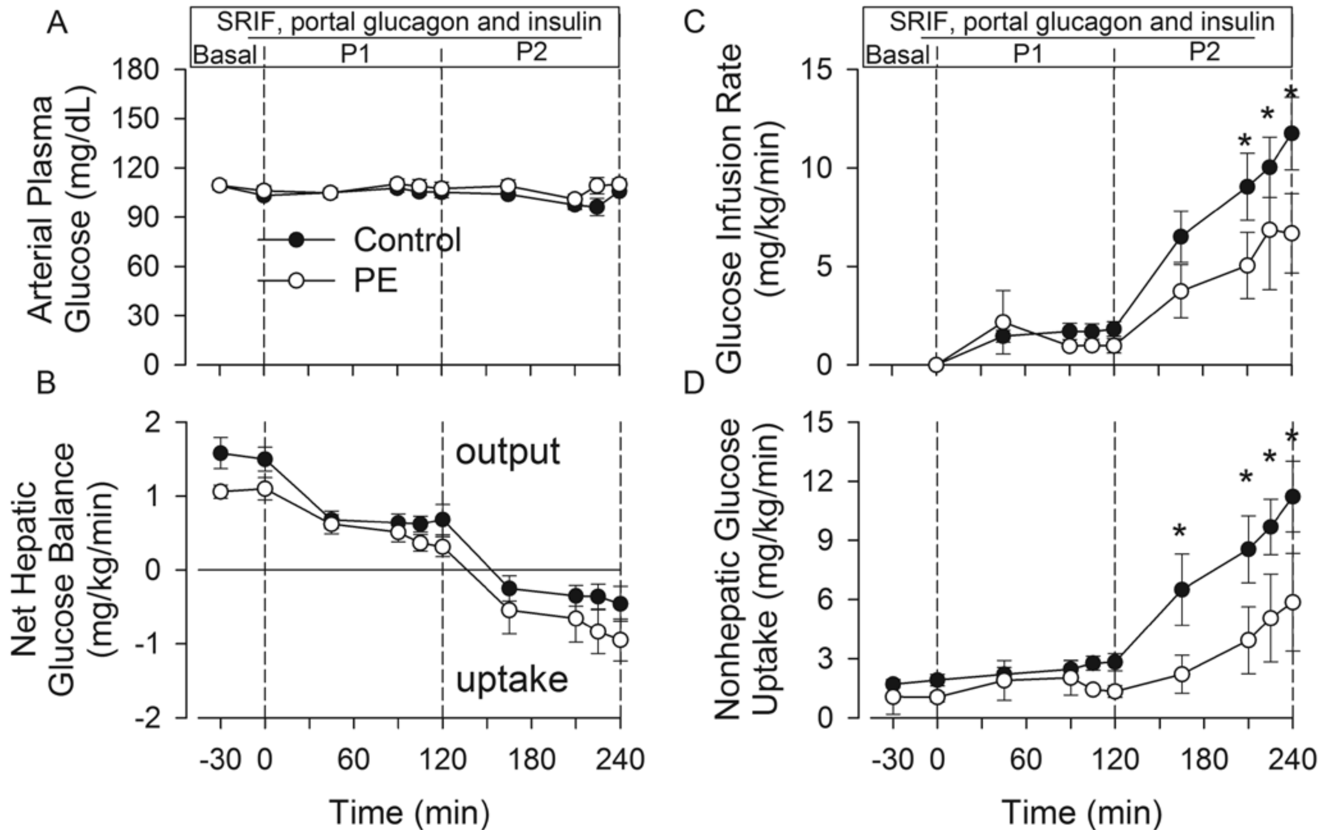

Figure 1. (A) Arterial plasma glucose concentration, (B) net hepatic glucose balance, (C) glucose infusion rate, and (D) nonhepatic glucose uptake in control (n = 6) and PE (n = 5) dogs. P1, 0–120 min; P2, 120–240 min; SRIF, somatostatin. Data are given as mean ± SEM. *, Value differs significantly (P < 0.05) between groups.

basal rates of secretion were approximately 30% greater in the PE dogs (294 ± 25 µU/kg/min) compared with the control group (223 ± 21 µU/kg/min; P < 0.05). In both groups, arterial plasma insulin concentrations increased slightly over basal values during P1 and then increased during P2 to approximately 4 times the basal level (Table 1).

Arterial plasma glucagon concentrations did not change significantly throughout the studies within groups, and there were no significant differences between groups at any time (Table 1). Arterial plasma cortisol concentrations remained at basal levels throughout the studies in both groups, with no significant differences between groups (data not shown).

Hepatic blood flow did not differ significantly between groups at any time, and it was stable over time within each group (Table 1).

Glucose data. The plasma glucose concentrations remained at basal levels (control group, 104 ± 3 mg/dL; PE group, 108 ± 3 mg/dL) during P1 and P2, and they did not differ significantly between groups at any time (Figure 1). The glucose infusion rates required to maintain euglycemia were 1.7 ± 0.5 and 1.0 ± 0.1 mg/kg/min during P1 and 9.9 ± 2.0 and 6.2 ± 1.1 mg/kg/min during P2 in the control and PE groups, respectively (P < 0.05 between groups during P2; Figure 1).

Net hepatic glucose balance did not differ significantly between groups during any period. In the basal state both groups were in a state of net hepatic glucose output (Figure 1). During P1, net hepatic glucose output in the 2 groups declined nearly 60% below the basal rate, and during P2 both groups switched to a low rate of net hepatic glucose uptake.

The rate of endogenous glucose production under basal conditions did not differ between control and PE dogs (2.8 ± 0.5 and 2.4 ± 2 mg/kg/min, respectively), and it fell similarly in both groups in response to hyperinsulinemia (Figure 2). The rate of glucose disappearance rose in both groups in response to hyperinsulinemia; the rates during P1 and P2 were approximately 35% greater (P = 0.05) in the control group than in the PE group.

Gluconeogenic precursors, NEFA, and β-hydroxybutyrate. Arterial concentrations and net hepatic balances of the gluconeogenic precursors lactate, alanine, and glycerol were similar in the 2 groups throughout the studies (Table 2). Net hepatic lactate uptake in both groups declined during P1 and P2, with a significant (P < 0.05) change in the control group during P2. In both groups, arterial glycerol concentrations declined significantly (P < 0.05) below baseline during P2, with a corresponding decrease in net hepatic glycerol uptake that reached statistical significance (P < 0.05) in the PE group.

Similar to glycerol, arterial concentrations of NEFA declined significantly (P < 0.05) in both groups in response to hyperinsulinemia; consequently the net hepatic NEFA uptake fell significantly (P < 0.05) below basal levels during P2 in both groups (Table 2). In both groups the arterial concentrations and net hepatic output of β-hydroxybutyrate declined in parallel with the supply of NEFA to the liver.

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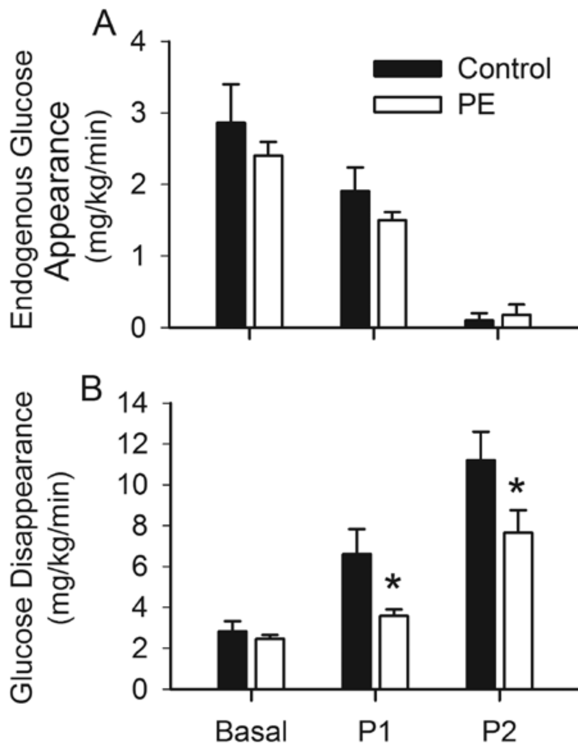

Figure 2. Tracer-determined rates of (A) endogenous glucose appearance and (B) glucose disappearance in control (n = 6) and PE (n = 5) dogs. P1, 0–120 min; P2, 120–240 min. Data are given as mean ± SEM. *, Value differs significantly ($P < 0.05$) between groups.

Discussion

The menstrual cycle in humans causes small changes in insulin sensitivity.[3,36] Likewise, the estrus cycle is a recognized cause of canine insulin resistance,[14,30] and the development of insulin-resistant diabetes mellitus associated with the estrus cycle in older dogs has been suggested to be a model of gestational diabetes in humans.[29,31] Consistent with this idea, the PE group demonstrated a marked loss of whole-body insulin sensitivity during P2, as evidenced by reductions in the glucose infusion rate, nonhepatic glucose uptake, and tracer-determined glucose disposal. Under hyperinsulinemic euglycemic conditions, the reduction in nonhepatic glucose uptake is primarily related to loss of muscle insulin sensitivity.[18]

Although proestrus and estrus are associated with insulin resistance, to our knowledge their effect on the liver's response to hyperinsulinemia had not been assessed previously. Insulin sensitivity during diestrus (45 d after estrus discharge) was previously evaluated in a group of 6 beagles by using the euglycemic hyperinsulinemic clamp technique, with the finding that the glucose infusion rate was reduced approximately 40% compared with the normal control group, consisting of 10 male and female beagles.[14] Although the findings of the previous study[14] are consistent with those in our current report, it is noteworthy that the single dose of insulin (1.5 mU/kg/min) used in those studies was more than sufficient to suppress hepatic glucose production completely in the dogs.[10,12,38] Maximal suppression of hepatic glucose output is evident at a point much lower on the insulin dose–response curve than are the responses of other insulin-sensitive tissues.

Table 2. Arterial blood lactate, alanine, glycerol, β-hydroxybutyrate, and nonesterified fatty acid concentrations (μmol/L) and net hepatic balances (μmol/kg/min) during the 3 experimental periods

	Basal	P1	P2
Lactate			
Arterial blood concentration			
control	623 ± 170	585 ± 115	752 ± 84
PE	496 ± 79	511 ± 39	534 ± 75
Net hepatic uptake			
control	2.7 ± 1.1	1.6 ± 1.9	0.6 ± 1.0[a]
PE	1.9 ± 1.7	1.4 ± 0.9	0.6 ± 0.6
Alanine			
Arterial blood concentration			
control	333 ± 76	324 ± 62	270 ± 38
PE	287 ± 30	271 ± 21	220 ± 14
Net hepatic uptake			
control	1.1 ± 0.6	1.2 ± 0.6	1.2 ± 0.6
PE	1.9 ± 0.3	2.1 ± 0.4	2.0 ± 0.4
Glycerol			
Arterial blood concentration			
control	93 ± 25	84 ± 29	57 ± 12[a]
PE	98 ± 6	84 ± 8	59 ± 14[a]
Net hepatic uptake			
control	0.9 ± 0.4	0.9 ± 0.5	0.7 ± 0.4
PE	1.4 ± 0.1	1.4 ± 0.2	1.0 ± 0.2[a]
Nonesterified fatty acids			
Arterial blood concentration			
control	955 ± 145	722 ± 192[a]	191 ± 69[a]
PE	1111 ± 80	743 ± 118[a]	213 ± 33[a]
Net hepatic uptake			
control	1.9 ± 0.5	1.1 ± 0.7	0.5 ± 0.4[a]
PE	2.0 ± 0.3	1.7 ± 0.4	0.5 ± 0.1[a]
β-hydroxybutyrate			
Arterial blood concentration			
control	57 ± 22	40 ± 16	7 ± 3[a]
PE	42 ± 13	23 ± 3	12 ± 1[a]
Net hepatic output			
control	0.9 ± 0.4	0.6 ± 0.3	0.1 ± 0.1[a]
PE	0.8 ± 0.1	0.4 ± 0.1[a]	0.1 ± 0.0[a]

Values are given as mean ± SEM (control, n = 6; PE, n = 5) of 3 sample times during the final 30 min of each period.
[a]Value significantly ($P < 0.05$) different from basal value within the same group. There were no significant differences between groups.

Thus, these previous findings provide no information about hepatic insulin sensitivity during diestrus. In addition, insulin was delivered through a peripheral vein, rather than the portal vein (its normal secretion route), in the previous study.[14] The direct effect of insulin on the liver is dominant over its indirect effects (primarily on fat tissue) in the control of hepatic glucose production under basal conditions.[12]

Our current report is the first to examine insulin sensitivity during estrus or diestrus, with delivery of insulin according to its physiologic route of secretion. In the current experiments, arterial plasma insulin concentrations increased modestly at the lower

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step of the 2-step hyperinsulinemic euglycemic clamp. This small increase in insulin was sufficient to decrease net hepatic glucose output and the rate of endogenous glucose production by 40% to 50% in both groups, consistent with our previous findings in normal male and female dogs.[5] Thus, the liver retained its insulin sensitivity during PE. We previously showed that the liver of the dog remains insulin-sensitive during late pregnancy, when progesterone concentrations are similar to those during PE.[10] However, to our knowledge, this current study represents both the first time that the response of the liver has been examined experimentally and the first time that the whole-body response to hyperinsulinemia has been so quantified in depth in a canine model during PE. Moreover, our use of mongrel dogs expands upon previous findings,[14] given that metabolic differences clearly exist among dogs of different sizes and breeds.[13,23-25]

Although our studies were not designed to examine the mechanism of the PE effects on peripheral insulin sensitivity, results of previous investigations strongly suggest that elevated progesterone concentrations explain at least part of PE's effect. In 3T3-L1 adipocytes, progesterone treatment was associated with reduced expression of IRS1, thus apparently contributing to impaired insulin-induced IRS1 phosphorylation, p85α–IRS1 association, and phosphorylation of Akt1 and Akt2.[42] Moreover, insulin-stimulated GLUT4 translocation was significantly suppressed in response to progesterone.[42] In ovariectomized cynomolgus monkeys, treatment with medroxyprogesterone acetate plus conjugated equine estrogen resulted in significantly less expression of GLUT4 in the rectus femoris muscle than did treatment with conjugated equine estrogen alone.[37] In addition, the muscle insulin receptor showed a trend toward decreased tyrosine phosphorylation in the group given medroxyprogesterone acetate.[37] The impairment of GLUT4 function at increased progesterone concentrations may explain the differential effect we observed between liver and whole-body insulin sensitivity in the PE dogs. The insulin-dependent GLUT4 mechanism is a key component of the glucose transport system in muscle and fat tissue, whereas the liver relies primarily on the low-affinity noninsulin-dependent GLUT2 glucose transporter.

Although glycerol and NEFA concentrations fell in parallel during P1 and P2, NEFA can undergo reesterification in the peripheral tissues, unlike glycerol.[39] Therefore glycerol concentrations provide a more robust indicator of suppression of lipolysis than NEFA concentrations do.[16,19] In the current study, the similarity of the glycerol data between groups indicates that the insulin resistance observed in the dogs in PE was limited primarily to glucose metabolism, with the suppression of lipolysis being retained during PE.

In conclusion, whole-body glucose disposal is impaired significantly during proestrus and estrus in mongrel dogs, whereas the response of the liver to hyperinsulinemia appears to be preserved. Because the liver is a key organ in glucose metabolism, its continued insulin responsiveness likely helps to prevent severe impairment of glucose metabolism during the estrus cycle. Indeed, the data suggest that a small reduction in nonhepatic glucose uptake during the basal period in the PE group was offset by a modest reduction in net hepatic glucose output, resulting in remarkably similar basal plasma glucose concentrations. Given the need to include both sexes in preclinical research, it is especially important that scientists understand the effect of the reproductive cycle on female animals in research models. The current data indicate that the stage of the cycle can substantially affect studies of glucose metabolism. The stage of the menstrual cycle apparently has much less of an effect on human glucose metabolism than is apparent in dogs during PE compared with anestrus.[3,11] Therefore metabolic studies predicated on normal muscle and whole-body insulin sensitivity should avoid using female canines while they are in proestrus or estrus. This accommodation increases research costs, given that the animals must either be replaced or housed until they enter anestrus. In contrast, the liver appears to retain its insulin sensitivity despite of the presence of PE.

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References

1. **Ayala JE, Bracy DP, McGuinness OP, Wasserman DH.** 2006. Considerations in the design of hyperinsulinemic euglycemic clamps in the conscious mouse. Diabetes **55:**390–397.
2. **Bigliardi E, Bresciani C, Callegari D, Di Ianni F, Morini G, Parmigiani E, Bianchi E.** 2014. Use of aglepristone for the treatment of P4 induced insulin resistance in dogs. J Vet Sci **15:**267–271.
3. **Bingley CA, Gitau R, Lovegrove JA.** 2008. Impact of menstrual cycle phase on insulin sensitivity measures and fasting lipids. Horm Metab Res **40:**901–906.
4. **Brackenridge A, Pearson ER, Shojaee-Moradie F, Hattersley AT, Russell-Jones D, Umpleby AM.** 2006. Contrasting insulin sensitivity of endogenous glucose production rate in subjects with hepatocyte nuclear factor-1β and -1α mutations. Diabetes **55:**405–411.
5. **Cherrington AD, Sindelar D, Edgerton D, Steiner K, McGuinness OP.** 2002. Physiological consequences of phasic insulin release in the normal animal. Diabetes **51 Suppl 1:**S103–S108.
6. **Clayton JA, Collins FS.** 2014. Policy: NIH to balance sex in cell and animal studies. Nature **509:**282–283.
7. **Coate KC, Kraft G, Shiota M, Smith MS, Farmer B, Neal DW, Williams P, Cherrington AD, Moore MC.** 2015. Chronic overeating impairs hepatic glucose uptake and disposition. Am J Physiol Endocrinol Metab **308:**E860–E867.
8. **Concannon PW.** 2011. Reproductive cycles of the domestic bitch. Anim Reprod Sci **124:**200–210.
9. **Connolly CC, Holste LC, Aglione LN, Neal DW, Lacy DB, Smith MS, Diamond MP, Cherrington AD, Chiasson JL.** 2000. Alterations in basal glucose metabolism during late pregnancy in the conscious dog. Am J Physiol Endocrinol Metab **279:**E1166–E1177.
10. **Connolly CC, Papa T, Smith MS, Lacy DB, Williams PE, Moore MC.** 2006. Hepatic and muscle insulin action during late pregnancy in the dog. Am J Physiol Regul Integr Comp Physiol **292:**R447–R452.
11. **Diamond MP, Jacob R, Connolly-Diamond M, DeFronzo RA.** 1993. Glucose metabolism during the menstrual cycle. Assessment with the euglycemic, hyperinsulinemic clamp. J Reprod Med **38:**417–421.
12. **Edgerton DS, Lautz M, Scott M, Everett CA, Stettler KM, Neal DW, Chu CA, Cherrington AD.** 2006. Insulin's direct effects on the liver dominate the control of hepatic glucose production. J Clin Invest **116:**521–527.
13. **Fleischer S, Sharkey M, Mealey K, Ostrander EA, Martinez M.** 2008. Pharmacogenetic and metabolic differences between dog breeds: their impact on canine medicine and the use of the dog as a preclinical animal model. AAPS J **10:**110–119.
14. **Fukuta H, Mori A, Urumuhan N, Lee P, Oda H, Saeki K, Kurishima M, Nozawa S, Mizutani H, Mishina S, Arai T, Sako T.** 2012. Characterization and comparison of insulin resistance induced by Cushing Syndrome or diestrus against healthy control dogs as determined by euglycemic- hyperinsulinemic glucose clamp profile glucose

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infusion rate using an artificial pancreas apparatus. J Vet Med Sci **74:**1527–1530.
15. **genSET gender in science.** [Internet]**.** 2016**.** The genSET Consensus report: recommendations for action on the gender dimension in science. [Cited 05 May 2016] Available at: http://www.genderinscience.org/index.php/consensus-seminars/recommendations-report.
16. **Groop LC, Bonadonna RC, Simonson DC, Petrides AS, Shank M, DeFronzo RA.** 1992. Effect of insulin on oxidative and nonoxidative pathways of free fatty acid metabolism in human obesity. Am J Physiol **263:**E79–E84.
17. **Issandou M, Bouillot A, Brusq JM, Forest MC, Grillot D, Guillard R, Martin S, Michiels C, Sulpice T, Daugan A.** 2009. Pharmacological inhibition of stearoyl-CoA desaturase 1 improves insulin sensitivity in insulin-resistant rat models. Eur J Pharmacol **618:**28–36.
18. **James DE, Jenkins AB, Kraegen EW.** 1985. Heterogeneity of insulin action in individual muscles in vivo: euglycemic clamp studies in rats. Am J Physiol **248:**E567–E574.
19. **Jensen MD, Caruso M, Heiling V, Miles JM.** 1989. Insulin regulation of lipolysis in nondiabetic and IDDM subjects. Diabetes **38:**1595–1601.
20. **Lalia AZ, Johnson ML, Jensen MD, Hames KC, Port JD, Lanza IR.** 2015. Effects of dietary n-3 fatty acids on hepatic and peripheral insulin sensitivity in insulin-resistant humans. Diabetes Care **38:**1228–1237.
21. **Mari A**. 2009. GLUTRAN: a Matlab toolbox for glucose tracer analysis. Padova (ITA): Institute of Biomedical Engineering, National Research Council.
22. **Mari A, Stojanovska L, Proietto J, Thorburn AW.** 2003. A circulatory model for calculating non-steady-state glucose fluxes. Validation and comparison with compartmental models. Comput Methods Programs Biomed **71:**269–281.
23. **Martinez MN, Antonovic L, Court M, Dacasto M, Fink-Gremmels J, Kukanich B, Locuson C, Mealey K, Myers MJ, Trepanier L.** 2013. Challenges in exploring the cytochrome P450 system as a source of variation in canine drug pharmacokinetics. Drug Metab Rev **45:**218–230.
24. **Meloni T, Comin A, Rota A, Peric T, Contri A, Veronesi MC.** 2014. IGF-I and NEFA concentrations in fetal fluids of term pregnancy dogs. Theriogenology **81:**1307–1311.
25. **Misbach C, Chetboul V, Concordet D, Medaille C, Gruet P, Speranza C, Hoffmann AC, Rocha A, Balouka D, Petit AM, Trehiou-Sechi E, Pouchelon JL, Lefebvre HP.** 2014. Basal plasma concentrations of routine variables and packed cell volume in clinically healthy adult small-sized dogs: effect of breed, body weight, age, and gender, and establishment of reference intervals. Vet Clin Pathol **43:**371–380.
26. **Moore MC, Coate KC, Winnick JJ, An Z, Cherrington AD.** 2012. Regulation of hepatic glucose uptake and storage in vivo. Adv Nutr **3:**286–294.
27. **Moore MC, Satake S, Baranowski B, Hsieh PS, Neal DW, Cherrington AD.** 2002. Effect of hepatic denervation on peripheral insulin sensitivity in conscious dogs. Am J Physiol Endocrinol Metab **282:**E286–E296.
28. **Moore MC, Smith MS, Sinha VP, Beals JM, Michael MD, Jacober SJ, Cherrington AD.** 2013. Novel PEGylated basal insulin LY2605541 has a preferential hepatic effect on glucose metabolism. Diabetes **63:**494–504.
29. **Nelson RW, Reusch CE.** 2014. Animal models of disease: classification and etiology of diabetes in dogs and cats. J Endocrinol **222:**T1–T9.
30. **Pöppl AG, Costa Valle S, Hilario Diaz Gonzalez F, de Castro Beck CA, Kucharski LC, Silveira Martins Da Silva R.** 2011. Estrus cycle effect on muscle tyrosine kinase activity in bitches. Vet Res Commun **36:**81–84.
31. **Pöppl AG, Mottin TS, Gonzalez FH.** 2013. Diabetes mellitus remission after resolution of inflammatory and progesterone-related conditions in bitches. Res Vet Sci **94:**471–473.
32. **Rand JS, Fleeman LM, Farrow HA, Appleton DJ, Lederer R.** 2004. Canine and feline diabetes mellitus: nature or nurture? J Nutr **134:**2072S–2080S.
33. **Renauld A, Gomez NV, Scaramal JD, Garrido D, Wanke MM.** 2003. Serum insulin, glucose and nonesterified fatty acids after administration of follicle-stimulating and luteinizing hormones in bitches. Medicina (B Aires) **63:**28–32.
34. **Ritz SA, Antle DM, Côté J, Deroy K, Fraleigh N, Messing K, Parent L, St-Pierre J, Vaillancourt C, Mergler D.** 2013. First steps for integrating sex and gender considerations into basic experimental biomedical research. FASEB J **28:**4–13.
35. **Scaramal JD, Renauld A, Gomez NV, Garrido D, Wanke MM, Marquez AG.** 1997. Natural estrous cycle in normal and diabetic bitches in relation to glucose and insulin tests. Medicina (B Aires) **57:**169–180.
36. **Schisterman EF, Mumford SL, Sjaarda LA.** 2013. Failure to consider the menstrual cycle phase may cause misinterpretation of clinical and research findings of cardiometabolic biomarkers in premenopausal women. Epidemiol Rev **36:**71–82.
37. **Shadoan MK, Zhang L, Wagner JD.** 2004. Effects of hormone therapy on insulin signaling proteins in skeletal muscle of cynomolgus monkeys. Steroids **69:**313–318.
38. **Sindelar DK, Chu CA, Venson P, Donahue EP, Neal DW, Cherrington AD.** 1998. Basal hepatic glucose production is regulated by the portal vein insulin concentration. Diabetes **47:**523–529.
39. **Steinberg D, Vaughan M, Margolis S, Price Wttao H, Pittman R.** 1961. Studies of triglyceride biosynthesis in homogenates of adipose tissue. J Biol Chem **236:**1631–1637.
40. **Tam CS, Xie W, Johnson WD, Cefalu WT, Redman LM, Ravussin E.** 2012. Defining insulin resistance from hyperinsulinemic-euglycemic clamps. Diabetes Care **35:**1605–1610.
41. **Tozzo E, Bhat G, Cheon K, Camacho RC.** 2015. Pioglitazone increases whole body insulin sensitivity in obese, insulin-resistant rhesus monkeys. PLoS One **10:**e0126642.
42. **Wada T, Hori S, Sugiyama M, Fujisawa E, Nakano T, Tsuneki H, Nagira K, Saito S, Sasaoka T.** 2010. Progesterone inhibits glucose uptake by affecting diverse steps of insulin signaling in 3T3-L1 adipocytes. Am J Physiol Endocrinol Metab **298:**E881–E888.

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