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Original Research

Long-Term Clinical Outcomes in Diabetic Rhesus Macaques (*Macaca mulatta*) Treated with Medroxyprogesterone Acetate for Endometriosis

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Depot medroxyprogesterone acetate (DMPA) is a common medical treatment for endometriosis in NHP. Because DMPA reportedly impairs glucoregulatory function in humans and rhesus macaques, as well as predisposes humans to diabetes mellitus (DM), we performed a retrospective study to further investigate its potential long-term clinical effects in animals with and without DM. Using a cohort of 29 rhesus macaques, we explored the hypotheses that DMPA treatment accelerates the onset of DM and that its use in rhesus macaques with endometriosis worsens clinical outcome measures (lifespan, body weight and body condition score). For both body weight and body condition score, a declining and statistically significant trend in mean values was evident as macaques developed either DM, or endometriosis or both. The addition of DMPA did not significantly alter this pattern. The presence of DM, endometriosis, or DMPA treatment statistically but not clinically significantly increased risk of death. Similarly, the presence of the 2 highly correlated variables endometriosis and DMPA treatment statistically but not clinically significantly increased the risk of incident DM. These results indicate that DMPA treatment was associated with worsening trends in lifespan and incident DM, however these trends did not achieve clinical significance in this cohort.

Abbreviations: BCS, body condition score; DMPA, depot medroxyprogesterone acetate; DM, diabetes mellitus;

Endometriosis and diabetes mellitus (DM) are relatively common conditions in primate colonies containing middle-aged and older rhesus macaques. The incidence of endometriosis in rhesus macaques varies between 20% and 31%, although the incidence in one cohort was as high as 45% in rhesus older than 20 y.[1,11,13,14,24,26,35,43] The actual incidence may be higher, given that asymptomatic NHP may go undetected or are identified through an incidental finding at necropsy. Type 2 DM occurs in many Old World NHP, including rhesus macaques. It most commonly occurs in older, obese primates and shares many key features with the human disease.[1,2,3,4,13,14,15,16,38,39] An estimated 21% of the United States population has diabetes, of which as much as 95% is due to type 2 DM.[3,4,5,8,9,12,23]

Endometriosis can be treated using surgical or medical methods. Surgical treatment can be complicated by widespread adhesions and hemorrhage. Furthermore, residual ectopic ovarian tissue may continue to provide hormonal support for the disease process.[1,11,14,25,35] Because of these factors, medical treatment to interrupt the hormonal cycle is a reasonable alternative. Depot medroxyprogesterone acetate (DMPA) is a synthetic progesterone derivative and is the most commonly used medical treatment for endometriosis in NHP.[11,15,24,26,27] DMPA suppresses ovulation and induces endometrial atrophy through its agonist activity at the progesterone receptor[31] and its capacity to inhibit the hypothalamic–pituitary–gonadal axis.[20] DMPA (150 mg IM once monthly) typically results in cessation of the reproductive cycle and reduction of clinical signs of endometriosis.[11,20] In addition, DMPA is able to bind to androgenic and glucocorticoid receptors[29,31] and, in humans, is associated with insulin resistance,[6,7,21,32,33,37] increased risk for type 2 DM in predisposed women[32] and deterioration of cardiometabolic parameters.[6,7,20,30,32,40,42]

In both humans and rhesus macaques, basal insulin and glucose levels do not change during the menstrual cycle. However during the luteal phase, when progesterone levels are high and estradiol levels are low, insulin sensitivity decreases markedly.[11,20] Recently, DMPA was shown to impair glucoregulatory function in rhesus macaques, most likely through the drug's affinity for glucocorticoid and sex steroid receptors.[11,29,33,37] The study demonstrated that decreased glucoregulatory function occurs in adult female rhesus macaques that are treated with DMPA, even if only for a short period of time.[11] However, whether DMPA treatment accelerates the onset of DM or worsens clinical outcomes is unclear. We performed a retrospective analysis in a cohort of 29 female rhesus macaques to determine whether DMPA treatment is associated with a worse long-term clinical outcome. Clinical outcome was assessed by using body condition score (BCS), body weight, time to death, and time to onset of DM.

Materials and Methods

Experimental animals. Subjects included 29 adult female rhesus macaques with an age range of 9.5 to 30 y. All animals were

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housed in social groups in an indoor colony at Yale University, an AAALAC-accredited facility. Housing and care were provided in accordance with the regulations of the Animal Welfare Act and recommendations of the *Guide for the Care and Use of Laboratory Animals*.[19] Subjects used for the current study were from various studies unrelated to the 2 primary spontaneous health conditions in our retrospective analysis. Animals were fed Old World Primate diet (no. 8714, Harlan Teklad, Indianapolis, IN) biscuits in addition to fresh fruit and vegetables daily. The amount of biscuit provided was determined individually according to body weight. Diabetic macaques received high-fiber biscuits (Harlan Teklad 7195 with 14.2% crude fiber). Three biscuits were given at time of insulin injection to ensure that the animal ate and to decrease the risk of hypoglycemia after insulin administration. Water was provided without restriction through an automated watering system or water bottles. The macaques were kept on a 12:12-h dark:light cycle.

Study design. Longitudinal data collection for each macaque diagnosed with endometriosis or DM (or both) was performed. Medical records were reviewed thoroughly to collect the information for specific time points in the macaque's medical history pertaining to the diagnosis and treatment of endometriosis and DM; these included the date of and age (in months) at diagnosis for endometriosis or DM, date of initiation of treatment for endometriosis or DM, body weight, BCS, and vital status (alive or dead). Data were collected at 2 points (6 and 12 mo) before diagnosis of endometriosis or DM and continued every 6 mo until each animal's end-point, that is, either the most current data (when the macaque was alive at the time of the current study) or the date of euthanasia or death. The time range during which data were collected varied from 1 y to more than 12 y. At conclusion of data collection, macaques were classified according to single or multiple disease combinations. Disease categories included macaques that were diabetics only (n = 3), those with endometriosis only (nondiabetics; n = 18), and those that had both DM and endometriosis (n = 8; Table 1). Of the 26 animals with endometriosis, 24 were treated with DMPA.

Clinical criteria. Animals underwent physical examination biannually while sedated for tuberculosis testing. Macaques were sedated (usually in the morning) with 7 to 10 mg/kg ketamine IM. The diagnostic and therapeutic guidelines for endometriosis and DM used over the 12-y span encompassing the scope of the retrospective study.

Endometriosis. Macaques were given a presumptive diagnosis of endometriosis when they were observed to have clinical signs associated with endometriosis, such as heavy bleeding, painful cycling, and palpable abdominal masses, as well as reproductive failure including abortions and failure to become pregnant. Animals exhibiting clinical signs consistent with endometriosis were all evaluated ultrasonographically. Ultrasound findings of cysts associated with the reproductive tract or aspiration of brown fluid from cysts was considered supportive evidence for endometriosis. Inconclusive ultrasound findings resulted in a presumptive diagnosis, with follow-up observation, clinical examination, and ultrasound testing until the diagnosis was confirmed or considered highly likely. Recurrent painful cycles with excessive bleeding were considered a justifiable basis on humane grounds for initiating DMPA therapy even when ultrasound examination was inconclusive. Laparascopic diagnosis is not available at our facility. Endometriosis was confirmed at necropsy in 6 macaques in this study. Presumptive or confirmed endometriosis was treated with monthly DMPA (40 mg IM) therapy. Published doses for treatment of endometriosis are 150 mg IM every 3 mo or 40 mg IM monthly.[11,27,35] In our hands, the 40-mg dose provides subjectively improved protection against breakthrough bleeding and pain, which can occur near the end of the 3-mo period. Three macaques in this study initially received leuprolide treatment (1.75 mg IM monthly) but were later transitioned to DMPA therapy for months to years prior to euthanasia (that is prior to the start period of data collection for this study). One macaque in this study received leuprolide for 4 y and did not receive DMPA at all before euthanasia.

DM. Preclinical detection of DM in the colony was aided by the measurement of blood glucose in macaques sedated for routine procedures (for example, tuberculosis testing) or any other clinical indication. Those animals with elevated blood glucose underwent further testing to obtain longitudinal blood glucose and fructosamine levels. Insulin therapy was instituted once a pattern of hyperglycemia (exceeding 100 mg/dL) accompanied by elevated fructosamine levels (greater than 250 µmol/L) was established. Insulin is administered at a starting dose of 1 unit twice daily. Short-acting insulin (Humulin 70/30, Lilly USA, Indianapolis, IN) is given in the morning, followed by long-acting insulin (Lantus, Sanofi–Aventis, Bridgewater, NJ) in the afternoon. Insulin doses were increased gradually by no more than 1 unit while the blood glucose was monitored daily. The final dose was achieved once blood glucose levels remained between 90 to 150 mg/dL for 3 consecutive days; fructosamine levels were checked approximately 3 wk afterward. Each diabetic animal was monitored on an ongoing basis by using a combination of variables (daily blood glucose, regular testing of fructosamine levels, food and water intake, clinical appearance and behavior, body weight, BCS, or institution of DMPA treatment or any other intervention that may affect glucoregulation) to determine when insulin doses should be altered. Although fructosamine levels below 230 µmol/L are considered reflect complete glucoregulation approximating that of a normal animal,[41] insulin doses were increased after consideration of all of the described variables, with fructosamine levels typically maintained in the 250- to 350-µmol/L range (data not shown).

Body weight and BCS. Body weight (in kilograms) was obtained by using a designated scale in each primate housing facility. The body condition scoring system used a 5-point grading scale in 0.5-point increments, with 5 denoting advanced obesity.[10,34] Note that the scoring of body condition during physical examinations is subjective despite the scoring guidelines, because multiple veterinarians perform the semiannual physical examinations.

Outcomes measures. In addition to the clinical measures listed above, various outcomes measures were defined for statistical analysis. Duration of disease was defined as the time (in months) between the diagnosis of disease (endometriosis or DM) and death or the end date of the study (if still living). For animals that were still living at the time of this study, in lieu of a time to death, the time from the initiation of treatment (DMPA or leuprolide) to the most current date was calculated as the duration of treatment. The time to treatment was the number of months between the diagnosis of endometriosis or DM and treatment initiation. The age (in months) of the macaque at euthanasia or death was recorded as the time to mortality. The age at onset of disease was

Table 1. Description of clinical parameters, DMPA treatment, and mortality in a cohort of 29 female rhesus macaques

	Mean age at onset or treatment (mo)	No. affected	Diagnostic groups		
			DM only	Endometriosis only	DM and endometriosis
DM	249.1	11	3	—	8[a]
Endometriosis	195.1	26	—	18	8
DMPA treatment	204.1	24	—	18	6
Death	300.3	9	1	4	4

DM, diabetes mellitus; DMPA, depot medroxyprogesterone acetate
[a]Of the 8 rhesus macaques with endometriosis and DM, diagnosis of endometriosis preceded that of DM in 7. In the remaining animals, DM was diagnosed 4 d prior to diagnosis of endometriosis.

the age of the macaque (in months) at the time of diagnosis of endometriosis or DM.

Statistical analysis. We hypothesized that DM results in shortened lifespan and worsened clinical outcomes measures (body weight and BCS) and that these effects are exacerbated by DMPA treatment in those macaques that have coincident endometriosis. We also hypothesized that DMPA treatment accelerates onset of DM.

The numbers of macaques within each disease category (DM only, endometriosis only, and both DM and endometriosis) and their mean age of onset (or treatment) for each clinical criteria (DM, endometriosis, DMPA treatment, or death) were tabulated. The median and interquartile range for decedent macaques in each of the sometimes overlapping clinical categories (DM only, endometriosis only, both DM and endometriosis, and both DM treatment and DMPA) were calculated also.

Because these characteristics are coded as present or absent (0 or 1), the Kendall τ[17] correlation coefficient was used to assess the association between DMPA and endometriosis. The interpretation of the Kendall τ is the same as the more familiar Pearson r used for parametric data. Highly correlated independent variables were entered in separate models to avoid problems with collinearity.

The age of DM onset was modeled with a Cox proportional hazards regression for the time-dependent variables of endometriosis and DMPA treatment. The age at death was similarly modeled, with the additional time-dependent disease of endometriosis. In these models, the diseases and treatment were considered absent until the time of initiation, thus marginal hazard ratios and their 95% confidence interval are presented to represent the effect of DMPA treatment and endometriosis over the complete follow-up period. For the repeated measures of body weight and BCS over the follow-up period, we applied linear mixed models[36] for the independent variables of DM, DMPA treatment, and age, all of which were time-varying; that is, updated at each observation. We accounted for the within-macaque correlation of measures with a first-order autoregressive covariance structure selected by the lowest corrected Akaike information criterion.[18] These models are robust to data missing at random. For the body-weight model, there were 29 macaques with 24 missing observations and 309 observed body weights, whereas for the BCS, there were 66 missing observations and 267 observed BCS. Adjusted (least square) means and 95% confidence intervals were compared by using posthoc *t* tests and were applied to produce graphs of body mass and BCS in diabetic and nondiabetic macaques divided by whether they had received DMPA or not. All statistical tests were 2-tailed, and a *P* value less than 0.05 was considered statistically significant. All analyses used SAS version 9.4 (SAS Institute, Cary, NC).

Results

Descriptive features of the dataset are given in Table 1. Of the 29 rhesus macaques in the cohort, 26 had endometriosis. Of these, 8 had concurrent diabetes mellitus. Endometriosis was typically diagnosed earlier (mean age, 195.1 mo [16.2 y]) than DM (mean age, 249.1 mo [20.7 y]), except for one animal in which the diagnosis of DM preceded that of endometriosis by 4 d. On average, DMPA treatment was instituted shortly (10 d) after diagnosis of endometriosis; 2 of the 26 animals with endometriosis were not treated with DMPA (one received leuprolide; the other macaque was not treated). By the end of the study, 9 animals in the cohort had died due to a variety of causes. Diabetic macaques treated with DMPA survived longest (median, 351.0 mo [29.2 y]) compared with those with DM and endometriosis (regardless of treatment; median, 317.5 mo [26.4 y]), endometriosis alone (median, 291.0 mo [24.2 y]), or DM alone (median, 300.0 mo [25.0 y]).

A Cox proportional hazards regression model was used to test whether the time-dependent variables of DM, endometriosis, and DMPA treatment altered age at death (Table 2). This model was selected to allow for multiple variables and changing disease status over time for each macaque. DM increased the risk of death approximately 2-fold, but this trend did not reach statistical significance. Similarly, the presence of either endometriosis or DMPA treatment resulted in nonsignificantly increased risk of death (hazard ratio, 3.18 and 1.72, respectively). DMPA and endometriosis were modeled separately given that they are highly correlated (Kendall τ correlation coefficient = 0.74, $P < 0.001$)—differences in hazard ratios for each reflected the effects of the 2 macaques not treated with DMPA on the model. A similar model was used to determine whether the time-dependent variables of endometriosis and DMPA treatment increased risk of incident DM. Because these 2 variables are highly correlated, they are both accompanied by an approximately 2-fold higher risk (hazard ratio, 2.06 and 2.78 respectively) of incident DM. This trend was not statistically significant.

Because DM and endometriosis are both longstanding conditions that occur in middle-aged to older animals, the clinical effects of DM alone or combined with DMPA treatment for endometriosis must be differentiated from those resulting from age alone. Therefore, for the repeated measures of body weight and BCS over the follow-up period, we applied linear mixed models for the age-adjusted predictors DM, endometriosis, and DMPA treatment (Table 3). For both body weight and BCS, a declining trend in mean values was evident as macaques developed

Table 2. Effect of clinical parameters on risk of death (associated with DM, endometriosis, DMPA treatment and combinations) and onset of incident DM (associated with DMPA treatment and endometriosis)

	Risk of death (n = 29)	
	Hazard ratio (95% CI)	P
	Model 1: Deaths = DM + Endometriosis	
DM	2.14 (0.49–9.33)	0.310
Endometriosis	3.18 (0.36–28.24)	0.300
	Model 2: Deaths = DM + DMPA treatment[a]	
DM	2.64 (0.58–12.00)	0.218
DMPA treatment	1.72 (0.36–8.26)	0.496
	Risk of incident DM[b] (n = 11)	
	Hazard ratio (95% CI)	P
Endometriosis	2.06 (0.44–9.57)	0.359
DMPA treatment	2.78 (0.73–10.59)	0.135

CI, confidence interval; DM, diabetes mellitus; DMPA, depot medroxyprogesterone acetate

The presence of DM, endometriosis, or DMPA treatment resulted in statistically insignificant increased risk of death. The presence of the 2 highly correlated variables endometriosis or DMPA treatment nonsignificantly increased the risk of incident DM in this population of 29 rhesus macaques.

[a]Because DMPA treatment occurred in 24 of the 26 macaques with endometriosis, these parameters are highly correlated (Kendall $\tau = 0.74$, $P < 0.001$) and cannot be modeled simultaneously[17]

[b]One of the 8 macaques developed DM prior to endometriosis and therefore was placed in the diabetes-only group.

either DM or endometriosis or both. Because DMPA treatment was highly correlated with endometriosis status, the same trend was noted for DM and DMPA treatment. One exception to this trend is the nonsignificantly higher body weight (but not BCS) in macaques with DM that were treated with DMPA. Animals lacking both DM and endometriosis had significantly greater body weights than those with either endometriosis ($P < 0.001$), or DM ($P = 0.004$), or the combination of both ($P < 0.001$). Similarly, macaques lacking both DM and DMPA treatment tended to have greater body weight than did animals with either condition singly (significant only in animals with DM [$P = 0.015$]) or in combination. Macaques without either DM or endometriosis had a significantly higher BCS than did animals with either DM alone ($P = 0.044$) or DM and endometriosis combined ($P = 0.040$). Similarly, macaques lacking both DM and DMPA treatment had significantly higher BCS than did animals treated with DMPA, regardless of whether they had DM ($P = 0.015$) or not ($P = 0.033$).

Discussion

The synthetic progesterone derivative DMPA is the most commonly used medical treatment for endometriosis in NHP.[11,26] In humans, DMPA is associated with insulin resistance,[21,25,28,32] increased risk for DM,[21,22,37] and deterioration of cardiometabolic parameters.[6,7,20,31,37] Recently, DMPA was shown to cause insulin resistance in rhesus macaques.[11] Because endometriosis and DM are common—and not infrequently coincident—conditions in primate colonies containing middle-aged and older rhesus macaques, the use of DMPA to treat endometriosis can occur in animals predisposed to or with DM. To examine whether DMPA treatment is associated with a worse long-term clinical outcome, we performed a retrospective analysis of 29 female rhesus macaques (*Macaca mulatta*) with DM, endometriosis, or both conditions. The majority of macaques with endometriosis had been treated with DMPA. Clinical outcome was assessed by using longitudinal analysis of BCS, body weight, time to mortality, and time to onset of DM.

Our results indicate that the major determinants of long-term clinical outcome were the primary conditions DM and endometriosis. The presence of either condition alone or in combination resulted in progressive (and statistically significant) declines in body weight and BCS and increased (but not significantly) risk of death. The addition of DMPA did not significantly change these patterns.

Interestingly, body weight was nonsignificantly higher in macaques treated with DMPA than those without DMPA treatment. In a previous NHP study, significant weight gain was not noted in 10 rhesus macaques treated with DMPA.[11] Although statistically insignificant, the trend toward increased body weight with DMPA treatment that occurred in our cohort (which was larger than that in the previous study)[11] is reminiscent of findings in humans. Multiple studies have shown that DMPA can cause weight gain in women.[2,3,4,5,6,21,25,28,29,32] In a clinical trial examining more than 3900 women treated with DMPA for as long as 7 y, as many as 5% of the women reported adverse reactions including weight changes (mostly weight gain).[2] Weight gain is a known risk factor for developing DM in humans and obesity in NHP. The increased body weight in our cohort was not accompanied by a similar increase in BCS, and this finding likely reflects the tendency for aging macaques to accumulate abdominal fat and lose muscle mass.

Congruent with studies showing that DM in humans increases risk of mortality,[3,4] the major determinant of declining clinical outcomes in this study is DM. As demonstrated in this group of rhesus macaques, DM, endometriosis, and DMPA treatment (a surrogate for endometriosis) each carried a 2- to 3 fold increased risk of death (Table 2), however these trends were not statistically significant, and sample sizes were very small. Although the presence of the correlated variables endometriosis and DMPA increased the risk of incident DM, this difference was not statistically significant in this cohort of 29 animals. Previous literature reports that DMPA causes insulin resistance rhesus macaques treated with DMPA.[11] From this perspective, DMPA treatment might be hypothesized to increase the risk of death and potentially worsen clinical outcomes in diabetics. However, in the current study population, the addition of DMPA did not substantially increase the risk of death or worsen clinical outcomes.

In our study cohort, diagnosis of endometriosis (mean time until diagnosis, 16.2 y) typically preceded diagnosis of DM (mean, 20.7 y), a pattern also noted in women. The age of diagnosis of endometriosis in women is during the reproductive years, typically between 15 to 49 y of age,[30] whereas the mean age at the diagnosis of diabetes is older (45 to 64 y).[8,9] The occurrence of basal hyperinsulinemia and or postprandial hyperinsulinemia in macaques older than 15 y is approximately 30%.[1,14,38] Delayed diagnosis of endometriosis in women is well recognized in the literature.[12,15,30] A similar trend has been noted in the NHP population, and in some cases, endometriosis is discovered at postmortem examination.[11]

Our results indicate that the major determinants of clinical outcomes among our rhesus macaques were the primary conditions

Table 3. Effect of DM, endometriosis, and DMPA treatment on the age-adjusted variables of body weight and body condition score

	Least-squares mean (lower–upper 95% confidence limits of the mean)	
Condition	Body weight (kg)	Body condition score (scale, 1–5)
Neither DM nor endometriosis	9.3 (8.7–9.9) [a,b,c]	3.5 (3.3–3.7)[a,b]
Neither DM nor DMPA	9.0 (8.4–9.6)[d]	3.5 (3.3–3.7)[c,d]
Not DM but endometriosis	8.5 (7.9–9.1)[a]	3.3 (3.2–3.4)
Not DM but DMPA	8.7 (8.1–9.3)	3.3 (3.1–3.4)[c]
DM but not endometriosis	8.2 (7.4–9.1)[b]	3.1 (2.7–3.5)[a]
DM but not DMPA	8.1 (7.2–9.0)[d]	3.2 (2.9–3.5)
DM and endometriosis	8.2 (7.5–9.0)[c]	3.1 (2.8–3.4)[b]
DM and DMPA	8.5 (7.7–9.3)	3.0 (2.7–3.3)[d]

A linear mixed model was used to assess the effects of diabetes mellitus (DM), endometriosis, and depot medroxyprogesterone acetate (DMPA) treatment on the repeated measures of body weight and body condition score throughout the reporting period. Comparisons were between states of either having or not having DM or endometriosis or DMPA treatment. These evolved sequentially within each macaque over time, starting with an animal that had neither DM nor endometriosis but developed one condition or both.

Regarding body weight, the group lacking both DM and endometriosis[a,b,c] have a significantly higher body weight than do macaques with either endometriosis ($P < 0.001$)[a], DM ($P = 0.004$),[b] or the combination of both ($P < 0.001$)[c]. Similarly, animals lacking both DM and DMPA treatment[d] tended to have higher body weights than do those with either condition singly (significant only in animals with DM [$P = 0.015$][d]) or in combination. Diabetic animals treated with DMPA tended to have higher body weights than those that were not treated, but this difference did not reach statistical significance.

Regarding body condition score (BCS), the group lacking both DM and endometriosis[a,b] had a significantly higher BCS than did animals with either DM alone ($P = 0.044$)[a] or with DM and endometriosis combined ($P = 0.040$)[b]. Similarly, macaques lacking both DM and DMPA treatment[c,d] had significantly higher BCS than did those treated with DMPA, regardless of whether they had DM ($P = 0.015$)[d] or not ($P = 0.033$)[c].

DM and endometriosis. The addition of DMPA did not significantly change these patterns. DMPA treatment was associated with nonsignificantly increased risk of mortality and incident DM, but our retrospective study cohort comprised only 29 animals. Conclusive evidence for clinically significant diabetogenic potential of DMPA is likely to require a prospective controlled study comparing DMPA and an alternative method of treating endometriosis.

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