

Sex Hormones, Insulin Sensitivity, and Diabetes Mellitus

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Abstract

Sex differences and the role of gonadal hormones in modulating insulin sensitivity and glucose tolerance are of increasing interest and importance because of the increasing prevalence of type 2 diabetes mellitus and the metabolic abnormalities associated with aging. Body composition is closely associated with insulin sensitivity, and increased body fat, particularly in the visceral compartment, is a risk factor for developing type 2 diabetes mellitus. Sex differences in body composition and/or insulin sensitivity are evident in humans throughout the lifespan. Ovarian hormones influence insulin sensitivity across the menstrual cycle, during pregnancy, and in the menopausal transition. Similarly, estrogens and progestins used for contraception and hormone replacement therapy affect glucoregulation. Nonhuman primates and humans have similar life histories and reproductive characteristics. As a result, nonhuman primates provide a valuable model for investigating factors related to insulin sensitivity. Studies of nonhuman primates have contributed significantly to our understanding of sex differences and the influence of sex steroids in this context. This brief review surveys present knowledge of the sex differences in body composition, insulin sensitivity, and risk for development of type 2 diabetes mellitus derived from studies in humans and nonhuman primates. The influences of endogenous and exogenous gonadal steroids are emphasized.

Key Words: body size and composition; diabetes mellitus; gonadal hormones; insulin sensitivity; reproductive condition; sex differences

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¹Abbreviations used in this article: BMI, body mass index; CEE, conjugated equine estrogens; DM2, type 2 diabetes mellitus; DXA, dual energy x-ray absorptiometry; FSIGTs, frequently sampled intravenous glucose tolerance tests; HRT, hormone replacement therapy; MPA, medroxyprogesterone acetate; NHANES III, Third National Health and Nutrition Examination Survey.

Introduction

The prevalence of type 2 diabetes mellitus (DM2¹) is increasing at a dramatic rate, and the economic costs of caring for patients with diabetic complications are high. The increase in DM2 is closely associated with the epidemic of obesity in industrialized countries. Reduced physical activity is a contributing factor as sedentary lifestyles become more common. Increased body fat, particularly in the visceral compartment, is a strong risk factor for the development of DM2. Advanced age is an additional risk factor, and people are living longer in this country and in many others. Elucidation of such risk factors will lead to interventions that can delay the onset or protect against the development of DM2.

Sex differences, as well as the effects of gonadal hormones and drugs that mimic or antagonize the effects of these hormones, are factors that may also have an impact on the development of DM2. Knowledge of the risk associated with these factors is important to individuals for making lifestyle choices and to medical providers counseling patients who are considering hormone use for contraception or as replacement therapy.

Animal models have provided important insights into the pathogenesis of human diseases and new therapeutic approaches. Nonhuman primates are particularly valuable in this context, because many of their life history and reproductive characteristics are very similar to those of our own species. This article provides an overview of available information on sex differences in risk for developing insulin resistance and the influences of endogenous and exogenous sex hormones, emphasizing studies of women and nonhuman primates. Because body composition is very closely associated with insulin sensitivity, the similarities and differences between nonhuman primates in terms of growth and body fat are also described.

Development and the Emergence of Sex Differences

Pregnancy in Old World nonhuman primates shares many characteristics with humans. The length of gestation is relatively long and typically culminates in a singleton birth. In rhesus monkeys, the average length of gestation is virtually identical for male and female fetuses, namely 165 days (Kemnitz 1994). As is the case in human fetal development, there is very little fat accretion during the first 5 to 6 mo of

gestation. Rhesus monkeys are born at approximately 5½ mo after conception and have very little body fat at birth. In contrast, human fetuses add body fat at an exponential rate during the third trimester of pregnancy (Southgate and Hey 1976).

The islet cells of the fetal rhesus monkey pancreas are clearly distinguishable by 60 days after conception, but the maximal growth rate of the pancreas occurs during later phases of gestation (Hoar and Monie 1981). The fetal testes are distinguishable and secrete testosterone by the seventh week after conception (Resko et al. 1980). Ovarian development in rhesus monkeys closely parallels human ovarian development in that mitotic activity in oogonia is maximal at 4 mo after conception and the quantity of oogonia and oocytes is similar to humans during development and at birth (Baker 1966).

Alterations in the intrauterine environment and resultant effects on fetal characteristics, such as birth weight, have been implicated as risk factors for adult diseases, including DM2, as part of the so-called “thrifty phenotype” hypothesis (Hales and Barker 1992). Extremes in birth weight are associated with an increased risk of DM2 later in life (Godfrey and Barker 2000). Decreased maternal insulin sensitivity is a common finding during the later stages of normal human and rhesus pregnancies and may be due to increasing levels of progesterone and other endocrine changes (Freinkel 1980). Maternal insulin resistance in humans allows preferential transfer of carbohydrates to the fetal compartment, supporting rapid growth of the fetus during the third trimester (Buchanan 1991). In humans, the variance in maternal insulin sensitivity accounts for significant portions of variance in birth weight and body composition of the newborn (Catalano and Kirwan 2001). Women with low body mass index (BMI¹) in early pregnancy tend to have smaller infants than heavier women, and weight gain during pregnancy is also positively correlated with birth weight of the infant (Shapiro et al. 2000). Low birth weight has been linked to insulin resistance and reduced pancreatic beta-cell responsiveness during childhood (Li et al. 2001). High birth weight also appears to confer an increased risk of diabetes. In Pima Indians, a population with a high risk of DM2, high birth weight is associated with maternal diabetes during pregnancy and an increased risk of DM2 in the offspring (McCance et al. 1994).

In some individuals, the inability to compensate completely for the insulin resistance during pregnancy may lead to gestational diabetes (Buchanan 1991). In pregnancies complicated by diabetes, the increased transfer of glucose and other metabolic fuels across the placenta to the fetus stimulates the fetal pancreas to secrete increased amounts of insulin in an attempt to control glycemia. In utero, insulin functions as a growth-promoting hormone, resulting in macrosomia (Farrell et al 1982). Additionally, an increase in fetal fat mass related to macrosomia occurs in diabetic pregnancies (Farrell et al. 1982; Jovanovic-Peterson et al. 1993). The effects of insulin on growth have been demonstrated experimentally in a nonhuman primate model by delivering

insulin directly to the rhesus fetus of nondiabetic mothers by an implanted minipump (Susa et al. 1979).

Similar to human pregnancies, maternal obesity in rhesus monkeys is associated with increased growth of the fetus. Pregnant rhesus monkeys with higher BMI at conception have exacerbated insulin resistance, indicated by higher fasting insulin concentration and lower glucose disappearance rates during an intravenous glucose tolerance test at day 125 of pregnancy, when compared with leaner pregnant females (Kemnitz et al. 1988). The incremental increase in insulin levels after intravenous glucose administration is directly proportional to BMI as shown in this study of more than 100 pregnancies, although some heavier gravidae are unable to compensate fully for the increased insulin resistance by increasing acute insulin secretion. Infants from these pregnancies are 12% heavier than expected for gestational age (Kemnitz et al. 1988). Whether this apparent increase in birth weight would have resulted in increased risk of DM2 in the offspring is unknown. Additionally, the association of extremes in birth weight with future risk of DM2 has not been studied in nonhuman primates.

Whether sex of the offspring has an impact on such changes during pregnancy and the resultant fetal consequences has not been fully explored in humans. Although male rhesus newborns weigh on average approximately 10% more than females (Goy and Kemnitz 1983; Kemnitz 1994), the birth weights of genetic females exposed to prenatal androgens are similar to birth weights of unexposed females (Goy and Kemnitz 1983). Additionally, sex differences in infant body composition have not been directly assessed in nonhuman primate models. The impact of sex differences in birth weight on future risk of DM2 has not been elucidated, because most studies of DM2 in rhesus monkeys have used males (Hamilton and Ciaccia 1978; Hansen and Bodkin 1986).

Juvenile and Peripubertal Period

Human females have greater fat mass and lesser fat free mass than males during the first 2 yr of life (Butte et al. 2000). There are few reports of sex differences in body composition and metabolism in immature nonhuman primates. Nutrient partitioning may differ between male and female infant baboons (Lewis et al. 1984). Male baboons gain more lean mass than females on a high calorie formula during the preweaning period whereas the increase in fat mass is approximately the same for both sexes. On a low calorie diet males gain more lean mass than the females, but females are more efficient at retaining calories in the form of fat (Lewis et al. 1984). The mechanism for this sex difference during early postnatal development and its implications for later development of DM2 are not clear.

Monkeys, like humans, have an extended juvenile growth period. Among prepubertal children, girls have greater total body fat and more subcutaneous fat as assessed by noninvasive imaging than boys, although visceral fat

content is not detectably different between the sexes at this stage of development (Arfai et al. 2002). Growth rates of male and female rhesus monkeys maintained on a standard laboratory diet are very similar during the juvenile period, but the small sex difference in absolute weight persists. Beginning at shortly before 2 yr of age, females undergo peripubertal acceleration of growth in body weight that is maximal at 30 mo of age and then declines as body weight plateaus during adulthood (Goy and Kemnitz 1983). The corresponding peripubertal increase in growth rate of males begins about 6 mo later than that of females and peaks at nearly 5 yr of age. It is both greater in magnitude and longer in duration than for females, resulting in the characteristically higher average body weights of males compared with females throughout adulthood (Goy and Kemnitz 1983; Hudson et al. 1996). Body composition and patterns of fat distribution have not yet been systematically studied during the juvenile period in monkeys.

It has been thought for many years that the timing of puberty is due in part to some metabolic signal to the central nervous system that body size and/or metabolic fuel reserves are sufficient to support reproduction (Cameron 1991). Because insulin levels often reflect energy reserve in the form of fat, insulin was thought to be a prime candidate for this role. Critical studies, however, have dissociated the changes in insulin levels from the activation of gonadotropin secretion, indicating strongly that insulin levels alone cannot be the critical link between peripheral metabolic status and reproductive hormone secretion (Cameron 1996).

A transient insulin resistance occurs during puberty in humans. During the transition from Tanner stage I to Tanner stage III, insulin sensitivity decreases by approximately 30% in both boys and girls (Goran and Gower 2001). This reduction is accompanied by increases in fasting glucose and insulin, and insulin increment in response to glucose. The decrease in insulin sensitivity is not statistically associated with changes in body fat, visceral fat, or levels of androgens or estradiol (Goran and Gower 2001). Androgen treatment in males with delayed puberty does not result in worsening insulin sensitivity, which provides additional evidence that the insulin resistance of puberty is not solely due to androgens in males (Saad et al. 2001; Wickman et al. 2002). Sex differences in leptin levels are evident during puberty and appear to be directly related to sex steroid concentrations. Girls exhibit higher levels of leptin than boys during puberty even after controlling for the increase in fat mass, and a marked rise in leptin during the transition from prepuberty to postpuberty is seen in females but not in males (Demerath et al. 1999). It is not clear how these differences in leptin levels during puberty translate to alterations in insulin sensitivity. Similar studies for nonhuman primates have not been reported.

Awareness of the metabolic changes during puberty is clinically important. Children who are already at risk for developing DM2 (e.g., related to obesity) may not be able to compensate fully for the additional insulin resistance of puberty (Goran et al. 2003).

Adulthood

For adults it is clear that insulin resistance is linked with obesity and particularly excessive abdominal body fat. Several studies demonstrate an increase in visceral fat mass in normal adult males compared with females, resulting in the typical "android" body type (Butte et al. 2000; Demerath et al. 1999; Despres et al. 2000; Hill et al. 1999; Lemieux et al. 1993; Snehalatha et al. 1997; Sumner et al. 2002). Analysis of data from the Insulin Resistance Atherosclerosis Study reveals that subcutaneous abdominal fat and visceral fat have both independent and combined effects on insulin sensitivity (Wagenknecht et al. 2003). Furthermore, this analysis shows that fat distribution also predicts insulin secretion. Therefore, sex differences in body composition are an important component of potential differences in insulin sensitivity in humans.

The sex difference in body weight of rhesus monkeys seen during adulthood also reflects differences in body composition. Evaluation of males and females representative of young adults (6-9 yr of age), middle-aged adults (15-19 yr), and older adults (26-30 yr) shows that males are heavier than females in all categories, and have greater crown-rump lengths and limb circumferences than females (Hudson et al. 1996). Males have greater absolute lean tissue mass than females, and females have a greater percentage of fat mass than males, as measured by dual energy x-ray absorptiometry (DXA¹). The increase in lean body mass of males is likely a consequence of the anabolic effects of testicular androgens, particularly on skeletal muscle. Testosterone propionate administered to gonadectomized male and female rhesus monkeys induces a rapid increase in body weight and indices of muscle mass (Kemnitz et al. 1988). Body fat mass is greatest during middle age for both sexes, and lean tissue mass declines in later adulthood (Hudson et al. 1996; Ramsey et al. 2000).

Fasting insulin levels and insulin increment to glucose challenge are typically highly correlated with the amount of body fat in healthy adult humans. A similar relation is found in laboratory-housed rhesus monkeys (Gresl et al. 2001; Hansen and Bodkin 1986; Kemnitz 1984; Kemnitz and Francken 1986) and in free-ranging rhesus monkeys (Schwartz et al. 1993).

Interestingly, unlike humans, there is no evidence for a sexually differentiated pattern of fat deposition in macaques (Pond and Mattacks 1987). Both sexes accumulate excess fat predominantly in the abdominal region, as evidenced by trunk and limb circumferences, skin fold thickness, and DXA; but differences in the visceral compartment have not been systematically studied (Hudson et al. 1996; Kemnitz et al. 1989b). This observation, which merits more detailed study, has implications for interpretation of data on sex differences in insulin sensitivity and glucose tolerance when monkeys are compared with humans. When sex differences in these end points are identified, they are less likely to be complicated by differences in fat distribution.

Adult female rhesus monkeys tend to have improved

glucose tolerance and greater insulin increments to intravenous glucose challenge than males (Kemnitz et al. 1989b; Ramsey et al. 2000). Use of frequently sampled intravenous glucose tolerance tests (FSIGTs¹), with evaluation of the data by minimal modeling, reveals enhanced insulin sensitivity and glucose effectiveness in females compared with males (Kemnitz et al. 1998). Importantly, disposition index, which reflects the combined influence of insulin secretion and insulin sensitivity, as well as the derived value of glucose effectiveness at zero insulin (reflecting the component of glucose effectiveness that is independent of insulin), are greater in females than in males.

These observations suggest that ovarian hormones contribute to the sex differences in glucoregulatory endpoints. Valdes and Elkind-Hirsch (1991) assessed insulin sensitivity in cycling women with FSIGTs and found a significant decrease in insulin sensitivity during the luteal phase of the menstrual cycle. To investigate whether this reduction occurs in monkeys, data from 14 FSIGTs conducted on females in the follicular phase of the menstrual cycle were compared with similar data from 13 FSIGTs from the luteal phase of the cycle (Kemnitz et al. 1998). Phases of the cycle were initially estimated by the typical changes in coloration of perineal skin and number of days from observed menstruation, and were subsequently confirmed by measurements of estradiol and progesterone in serum samples collected on the day of the FSIGTs. Plasma estrogen levels were approximately three-fold higher in the follicular phase compared with the luteal phase, whereas luteal phase progesterone levels were approximately 100-fold greater than follicular phase values. Results of the FSIGTs clearly indicate that both insulin sensitivity and disposition index are greater during the follicular than the luteal phase of the menstrual cycle. The reduced insulin secretion and action during the luteal phase are more likely due to elevated progesterone levels rather than waning estrogen levels (see below).

It is not likely that the sex differences observed in glucoregulatory end points are from testicular androgens. Biliar and colleagues (1987) treated intact cycling female rhesus monkeys with androstenedione for as long as 4½ yr, and they saw no change in circulating basal insulin, glucose tolerance, or plasma C-peptide concentration. Androstenedione can be aromatized to estrogen, which could explain the lack of effect on insulin sensitivity in the aforementioned study. In the study by Wickman and colleagues (2002), cited above, testosterone therapy given to boys with delayed puberty had no impact on insulin concentration. However, when testosterone was administered with an aromatase inhibitor, insulin concentrations decreased. This result suggests that androgens do not directly impair insulin sensitivity. Furthermore, Tyagi and colleagues (1999) treated intact male rhesus monkeys with 50 mg of testosterone enanthate bimonthly for nearly 3 yr and saw no change in glucose tolerance, although fasting insulin values decreased significantly after 27 mo of treatment and returned to baseline values within 3 mo after withdrawal of

treatment. Islet cells of baboons contain receptors for estrogen and progesterin, but not testosterone (Winborn et al. 1983, 1987a, 1987b), suggesting a direct role for ovarian but not testicular hormones in modulating insulin secretion. In further support of the hypothesis, female-to-male transsexual individuals treated with testosterone injections show no or only modest decrements in insulin sensitivity (Elbers et al. 2003; Polderman et al. 1994). The insulin resistance often seen in hyperandrogenic women with the polycystic ovary syndrome is associated with a more complex pathophysiology, as discussed elsewhere in this issue (Abbott et al. 2004). In conclusion, endogenous progestins impair insulin sensitivity, and endogenous estrogens and androgens appear to have minimal effects on glucoregulation. The basis for the sex differences in insulin sensitivity may therefore be more strongly associated with body composition and fat distribution, although this possibility merits further investigation.

Insulin resistance during pregnancy is discussed above in the context of an altered intrauterine environment. The effects of this insulin resistant state are also important for maternal health. Women who develop gestational diabetes, presumably from the inability to compensate for increased insulin resistance, have a significantly increased risk for future development of DM2. Prepregnancy BMI, blood glucose at diagnosis, and persistent hyperglycemia 2 mo after giving birth positively correlate with future diabetes risk (Coustan et al. 1993; Damm et al. 1992). To our knowledge, this phenomenon has not been studied in depth in nonhuman primates.

Later Life

Insulin resistance is often seen in older people, and there is increased risk of developing DM2 in later life (Muller et al. 1996). The potential role of changing levels of bioavailable gonadal hormones in this context is unclear. There is an increase in glucose and insulin levels associated with the menopausal transition (Carr 2003), which may be related to changes in body composition (Poehlman et al. 1995). Low testosterone levels in men and high testosterone levels in women predict insulin resistance and DM2 in older adults (Oh et al. 2002).

There are few reported studies of glucose tolerance and insulin sensitivity in older monkeys, largely due to the limited availability of these animals. Ramsey and colleagues (2000) compared 28- to 37-yr-old female rhesus monkeys and 23- to 37-yr-old males with younger adults. The older females were postmenopausal based on lack of menstruation and cyclic changes in sex skin coloration for at least 1 yr before study. Males had greater lean body mass than females, but there was not a significant sex difference in fat mass. Across all age groups females had greater values for glucose tolerance, and for acute and second phase insulin responses to glucose challenge, than males. There was a trend toward lower insulin responses in both older groups.

Older animals of both sexes had lower metabolic rates and decreased levels of physical activity compared with younger adults.

Contraceptive Agents

The effects of oral contraceptive agents on carbohydrate metabolism have been extensively studied in women, and this area continues to be actively investigated as new dosages and combinations of synthetic agents are evaluated and comparisons of different routes of administration are made. In general, studies of women indicate that the use of newer oral contraceptive formulations have minimal deleterious effects on glucose tolerance and insulin sensitivity and are not associated with an increased risk for development of DM2 (Adams et al. 1980; Chasan-Taber et al. 1997; Godsland and Crook 1994; Kim et al. 2002; Rimm et al. 1992; Vela and Yen 1969). As lower doses of estrogen and newer progestins with less androgenic activity and more specificity are coming into use, the side effects of oral contraceptives are further reduced (Godsland et al. 1990; Ludicke et al. 2002). Although oral, low-dose, progestin-only contraceptives have minimal metabolic effects (Godsland et al. 1990, 1992), parenteral administration of medroxyprogesterone is associated with worsening glucohomeostasis (Amatayakul et al. 1980), particularly with longer duration of use (Liew et al. 1985). Furthermore, Kim and colleagues (2001) reported an increased risk of developing DM2 in Navajo women using depot medroxyprogesterone for contraception. Differences in progestin formulation and route of administration may therefore be important variables with regard to metabolic effects. To our knowledge, newer transdermal contraceptives have not been studied in this context.

Surprisingly little work in this area using nonhuman primates has been published, and most of this was published more than 20 yr ago (Beck 1969, 1977; Beck et al. 1975; Goldzieher et al. 1978). A survey of glucose tolerance in zoo-housed orangutans revealed no difference between females with contraceptive implants and untreated females (Gresl et al. 2000).

Hormone Replacement Therapy

Although testosterone replacement therapy in hypogonadal men appears to have either a neutral (Tripathy et al. 1998) or a beneficial effect on insulin sensitivity (Simon et al. 2001), numerous studies of hormone replacement therapy (HRT¹) in women have yielded variable results. Variations in the hormonal composition, doses, route of administration, and differences in study design likely account for the variable influence on insulin sensitivity.

HRT appears to attenuate the weight gain associated with menopause. Haarbo and colleagues (1991) reported that continuous oral estradiol either alone or in combination with cyproterone acetate or levonorgestrel prevents an in-

crease in abdominal fat mass assessed by DXA in naturally postmenopausal Danish women compared with placebo. Similar findings have been reported with oral conjugated equine estrogen (CEE¹) alone or in combination with medroxyprogesterone acetate (MPA¹) (Reubinoff et al. 1995). Mattiasson and colleagues (2002) performed a more detailed analysis of body composition with computed tomography and found a reduction in visceral fat mass in postmenopausal women treated with estradiol and cyclic MPA.

Despite the attenuation of weight gain, several studies have reported worsening insulin sensitivity with oral HRT (Ryan et al. 2002; Soranna et al. 2002). In the Postmenopausal Estrogen/Progestin Interventions trial, HRT with continuous CEE alone or in combination with MPA or micronized progesterone was associated with an increase in glucose levels obtained 2 hr after an oral glucose load, whereas fasting glucose levels decreased slightly in the treatment groups (Writing Group for the PEPI Trial 1995). Similar results have been reported in American Indian women (Zhang et al. 2002). As with oral contraceptives, the dose of estrogen use may influence metabolic parameters. Lobo and colleagues (2001) reported that lower doses of CEE alone or combined with MPA have beneficial effects on lipid status, but only minimal dose-response changes are seen in carbohydrate metabolism. Additional studies examining dose-response are warranted.

Different formulations of estrogen may have varying effects on glucohomeostasis. Studies using estradiol have yielded conflicting results. The study by Soranna and colleagues (2002) cited above reported reduced insulin sensitivity in subjects randomized to continuous oral estradiol alone or in combination with dydrogesterone without concurrent changes in BMI or abdominal fat mass as estimated by the waist:hip ratio. However, in another study, insulin sensitivity was enhanced with oral estradiol, but the effect was negated when oral norethindrone acetate was added (Spencer et al. 2000). The latter result suggests that the addition of certain progestogens may be an important factor in reducing insulin sensitivity. This notion is consistent with the findings of decreased insulin sensitivity during the luteal phase of the menstrual cycle when progesterone levels are high. In further support of this hypothesis, oral estradiol in combination with MPA, but not estradiol alone, results in reduced insulin sensitivity in women with premature ovarian failure (Elkind-Hirsch et al. 1993). In other studies, treatment with CEE alone results in improved glucohomeostasis, and these effects are attenuated by the addition of a progestin (Lindheim et al. 1993; Lobo et al. 1994).

The route of administration of HRT may also influence glucohomeostasis because transdermal steroids do not undergo first pass metabolism by the liver (Ansbacher 2001). Transdermal estradiol tends to have a neutral or beneficial effect on insulin sensitivity in normoinsulinemic women (Cucinelli et al. 1999; Duncan et al. 1999; Godsland et al. 1993; Raudaskoski et al. 1999; Spencer et al. 2000). The addition of oral progestins with low androgenic potential

(Duncan et al. 1999; Godsland et al. 1993) or transdermal progestins (Stevenson et al. 1993) appear to have little effect on insulin sensitivity.

HRT appears to have a neutral or beneficial effect on glucose metabolism in women who have insulin resistance or diabetes. In the study by Cucinelli and colleagues (1999) cited above, a subgroup of hyperinsulinemic women had a significant reduction in plasma insulin and insulin area under the curve with transdermal estradiol alone or with combination therapy. Other studies have reported similar findings in insulin-resistant women treated with oral continuous combined HRT with CEE and MPA (Saglam et al. 2002; Sumino et al. 2003). Based on the Third National Health and Nutrition Examination Survey (NHANES III¹), women with DM2 on HRT have lower fasting glucose levels than never users (Crespo et al. 2002). Another evaluation of NHANES III data shows a neutral effect on fasting glucose, and A1c, a marker of glucose control, although post-challenge glucoses are slightly higher in HRT users (Triusu et al. 2000). The two evaluations of the NHANES III used different inclusion criteria, therefore the sample sizes were different. Araujo and colleagues (2002) reported no deleterious effects on glucose metabolism in women with DM2 treated with oral CEE or transdermal estradiol combined with micronized progesterone. Additional studies show improvements in measures of glucose control in women with DM2 treated with oral estradiol alone (Andersson et al. 1997) and CEE alone (Friday et al. 2001) or in combination with MPA (Manning et al. 2001). Other small studies show improved gluco-regulation in women with DM2 taking oral or transdermal estradiol as part of combination HRT (Borissova et al. 2002; Darko et al. 2001).

Despite this apparent beneficial effect on glucose metabolism, women with diabetes mellitus who used HRT had an increased risk of ischemic heart disease, myocardial infarction, and death compared with never users in a prospective observational study of Danish nurses (Lokkegaard et al. 2003). Although subjects with known cardiac disease at baseline were excluded, it is conceivable that women with asymptomatic heart disease participated in the study. In this case, the findings from this study are consistent with increased thrombotic events reported in secondary prevention trials (Hulley et al. 1998). A previous case control study showed no increased risk of myocardial infarction in diabetic women using HRT (Kaplan et al. 1998).

HRT is associated with a neutral or reduced risk of developing diabetes. The Rancho Bernardo Heart and Chronic Disease Study reported no altered risk of developing diabetes in women on HRT in a community-based cohort of postmenopausal Caucasian women (Gabal et al. 1997). Similar findings were demonstrated in the prospective study of a subgroup of mainly white, healthy postmenopausal women in the Nurses' Health Study (Manson et al. 1992). In the Heart and Estrogen/Progestin Replacement Study, combined HRT with CEE and MPA is associated with a reduced risk of developing diabetes in postmenopausal women with coronary disease (Kanaya et al. 2003).

The North American Menopause Society recommends that women with insulin resistance or diabetes who desire HRT for menopausal symptom relief should use continuous-cyclic HRT with a low-dose, oral micronized progesterone (NAMS 2003). Alternatively, vaginal or intrauterine progesterone may decrease the adverse metabolic effects associated with progesterone use.

Similar to HRT, the few examples in the literature of studies that have investigated the effects of the selective estrogen receptor modulator raloxifene provide inconsistent results with both neutral (Cucinelli et al. 2002) and negative effects on gluco-regulation in nondiabetic postmenopausal women (Lee et al. 2003). Additional studies have reported neutral effects on gluco-regulation in women with DM2 (Andersson et al. 2002; Barrett-Connor et al. 2003). Additional studies are needed in this regard.

Biases related to study design have made it difficult to draw conclusions about the risks and benefits of HRT in women as evidenced by the recent Women's Health Initiative study (Rossouw et al. 2002). Therefore, studies of HRT in nonhuman primates may provide useful information because many biases (e.g., self-selection) can be minimized. However, most studies of HRT in monkeys have been in the context of surgical menopause in younger monkeys, mimicking the postmenopausal condition. Whether surgical withdrawal of ovarian hormones, particularly in young monkeys, is an entirely appropriate model of menopause is a matter of some debate (Bellino and Wise 2003), but studies using this approach have generated useful data.

Middle-aged ovariectomized rhesus monkeys were studied during periods of treatment with estradiol alone, progesterone alone, and estradiol plus progesterone (Kemnitz et al. 1989a). In this study, there were no detectable effects of hormone treatment on fasting glucose levels or glucose tolerance during intravenous glucose tolerance tests, but progesterone treatment significantly increased insulin levels. Both fasting insulin concentration and insulin response to glucose challenge were increased by approximately 50% during treatments with progesterone alone or with the combination of estradiol plus progesterone.

Wagner and colleagues (1996) studied ovariectomized female cynomolgus monkeys fed a lipid-lowering diet and given no HRT, CEE alone, or CEE combined with MPA for 30 mo. Monkeys receiving combined hormone replacement had significantly higher fasting glucose and insulin levels and higher insulin responses to a glucose challenge compared with controls or those given estrogen alone. Monkeys given estrogen-only therapy had lower body weights, lower measures of abdominal adiposity, and decreased serum androgen concentrations.

Wagner and colleagues (1998) subsequently studied ovariectomized adult female cynomolgus monkeys fed a moderately atherogenic diet, with one of the following three treatments added to the diet: no treatment (control), CEE alone, or CEE combined with nomegestrol acetate, a progestin without androgenic activity. Insulin sensitivity was assessed after 10 wk of treatment. In contrast to their studies

of CEE and MPA, CEE plus nomegestrol treatment, although reducing the insulin sensitivity to less than that of the CEE only group, did not reduce the insulin sensitivity index to less than that of control monkeys.

Cefalu and colleagues (1994) studied surgically postmenopausal cynomolgus monkeys that were fed a moderately atherogenic diet for 12 wk and received no treatment (control), CEE, MPA, a combination CEE and MPA, or tamoxifen. Compared with control animals or CEE alone, insulin sensitivity was significantly decreased in animals treated with MPA or CEE combined with MPA. Although insulin sensitivity was decreased in the tamoxifen-treated animals, the difference was not statistically significant compared with the control or CEE-treated animals. These results suggest that progestins alone or in combination with estrogens can induce insulin resistance in postmenopausal monkeys.

More recently, Shadoan and colleagues (2003) investigated the effects of tibolone (a synthetic steroidal agent with estrogenic, progestogenic, and androgenic activity) on body weight, body composition, and fasting carbohydrate measures in surgically postmenopausal cynomolgus monkeys that were compared with those receiving CEE with and without MPA. Compared with controls, body weight significantly increased and abdominal soft tissue mass was greater in all but the CEE-treated group. They concluded that HRT with CEE combined with MPA or tibolone results in greater body weight, abdominal soft tissue, and insulin resistance compared with control-treated monkeys.

To summarize, although the human studies of oral HRT are inconsistent, HRT is not associated with an increased risk of DM2, and transdermal estrogens appear to have little impact on insulin sensitivity. HRT has neutral to beneficial effects on glucoregulation in women with diabetes, but additional studies are needed to investigate whether HRT alters cardiac endpoints in this population. The nonhuman primate studies indicate that deleterious effects of HRT on glucoregulation may be related more to the progestin than to the estrogen. Additional investigations of the effect of selective estrogen receptor modulators on insulin sensitivity are warranted.

Conclusion

There are striking similarities among macaques, baboons, and humans in body composition, insulin sensitivity, and development of DM2, which supports the use of the nonhuman primate model in this context. The nonhuman primate model holds particular value for assessing sex differences in factors related to insulin sensitivity as well as benefits and risks of hormonal treatment of adults during later life. In light of the recent reports of deleterious consequences of HRT on cardiovascular health in women, additional studies of nonhuman primates that explore new dosages, combinations, and routes of administration of hormones and selective estrogen receptor modulators are needed.

References

- Abbott DH, Foong SC, Barnett DK, Dumesic DA. 2004. Nonhuman primates contribute unique understanding to anovulatory infertility in women. *ILAR J* 45:116-131.
- Adams PW, Godsland I, Melrose J, Nithyananthan R, Oakley NW, Seed M, Wynn V. 1980. The influence on oral contraceptive formulation on carbohydrate and lipid metabolism. *J Pharmacother* 3:54-63.
- Amatayakul K, Sivassomboon B, Singkamani R. 1980. Effects of medroxyprogesterone acetate on serum lipids, protein, glucose tolerance and liver function in Thai women. *Contraception* 21:283-297.
- Andersson B, Johannsson G, Holm G, Bengtsson BA, Sashegyi A, Pavo I, Mason T, Anderson PW. 2002. Raloxifene does not affect insulin sensitivity or glycemic control in postmenopausal women with type 2 diabetes mellitus: A randomized clinical trial. *J Clin Endocrinol Metab* 87:122-128.
- Andersson B, Mattsson LA, Hahn L, Marin P, Lapidus L, Holm G, Bengtsson BA, Bjornorp P. 1997. Estrogen replacement therapy decreases hyperandrogenicity and improves glucose homeostasis and plasma lipids in postmenopausal women with noninsulin-dependent diabetes mellitus. *J Clin Endocrinol Metab* 82:638-643.
- Ansbacher R. 2001. The pharmacokinetics and efficacy of different estrogens are not equivalent. *Am J Obstet Gynecol* 184:255-263.
- Araujo DA, Farias ML, Andrade AT. 2002. Effects of transdermal and oral estrogen replacement on lipids and glucose metabolism in postmenopausal women with type 2 diabetes mellitus. *Climacteric* 5:286-292.
- Arfai K, Pitukcheewanont PD, Goran MI, Tavare CJ, Heller L, Gilsanz V. 2002. Bone, muscle, and fat: Sex-related differences in prepubertal children. *Radiology* 224:338-344.
- Baker TG. 1966. A quantitative and cytological study of oogenesis in the rhesus monkey. *J Anat* 100:761-776.
- Barrett-Connor E, Ensrud KE, Harper K, Mason TM, Sashegyi A, Krueger KA, Anderson PW. 2003. Post hoc analysis of data from the Multiple Outcomes of Raloxifene Evaluation (MORE) trial on the effects of three years of raloxifene treatment on glycemic control and cardiovascular disease risk factors in women with and without type 2 diabetes. *Clin Ther* 25:919-930.
- Beck P. 1969. Progestin enhancement of the plasma insulin response to glucose in rhesus monkeys. *Diabetes* 18:146-152.
- Beck P. 1977. Effect of progestins on glucose and lipid metabolism. *Ann N Y Acad Sci* 286:434-445.
- Beck P, Venable RL, Hoff DL. 1975. Mutual modification of glucose-stimulated serum insulin responses in female rhesus monkeys by ethinyl estradiol and nortestosterone derivatives. *J Clin Endocrinol* 41:44-53.
- Bellino FL, Wise PM. 2003. Nonhuman primate models of menopause workshop. *Biol Reprod* 68:10-18.
- Billiar RB, Richardson D, Schwartz R, Posner B, Little B. 1987. Effect of chronically elevated androgen or estrogen on the glucose tolerance test and insulin response in female rhesus monkeys. *Am J Obstet Gynecol* 157:1297-1302.
- Borissova AM, Tankova T, Kamenova P, Dakovska L, Kovacheva R, Kirilov G, Genov N, Milcheva B, Koev D. 2002. Effect of hormone replacement therapy on insulin secretion and insulin sensitivity in postmenopausal diabetic women. *Gynecol Endocrinol* 16:67-74.
- Buchanan TA. 1991. Glucose metabolism during pregnancy: Normal physiology and implications for diabetes mellitus. *Isr J Med Sci* 27:432-441.
- Butte NF, Hopkinson JM, Wong WW, Smith EO, Ellis KJ. 2000. Body composition during the first 2 years of life: An updated reference. *Pediatr Res* 47:578-585.
- Cameron JL. 1991. Metabolic cues for the onset of puberty. *Horm Res* 36:97-103.
- Cameron JL. 1996. Regulation of reproductive hormone secretion in primates by short-term changes in nutrition. *Rev Reprod* 1:117-126.
- Carr MC. 2003. The emergence of the metabolic syndrome with menopause. *J Clin Endocrinol Metab* 88:2404-2411.

- Catalano PM, Kirwan JP. 2001. Maternal factors that determine neonatal size and body fat. *Curr Diab Rep* 1:71-77.
- Cefalu WT, Wagner JD, Bell-Farrow AD, Wang ZQ, Adams MR, Toffolo G, Cobelli C. 1994. The effects of hormonal replacement therapy on insulin sensitivity in surgically postmenopausal cynomolgus monkeys (*Macaca fascicularis*). *Am J Obstet Gynecol* 171:440-445.
- Chasan-Taber L, Willett WC, Stampfer MJ, Hunter DJ, Colditz GA, Spiegelman D, Manson JE. 1997. A prospective study of oral contraceptives and NIDDM among US women. *Diabetes Care* 20:330-335.
- Coustan DR, Carpenter MW, O'Sullivan PS, Carr SR. 1993. Gestational diabetes: Predictors of subsequent disordered glucose metabolism. *Am J Obstet Gynecol* 168:1139-1145.
- Crespo CJ, Smit E, Snelling A, Sempos CT, Andersen RE. 2002. Hormone replacement therapy and its relationship to lipid and glucose metabolism in diabetic and nondiabetic postmenopausal women: Results from the Third National Health and Nutrition Examination Survey (NHANES III). *Diabetes Care* 25:1675-1680.
- Cucinelli F, Paparella P, Soranna L, Barini A, Cinque B, Mancuso S, Lanzone A. 1999. Differential effect of transdermal estrogen plus progestagen replacement therapy on insulin metabolism in postmenopausal women: Relation to their insulinemic secretion. *Eur J Endocrinol* 140:215-223.
- Cucinelli F, Soranna L, Romualdi D, Muzj G, Mancuso S, Lanzone A. 2002. The effect of raloxifene on glyco-insulinemic homeostasis in healthy postmenopausal women: A randomized placebo-controlled study. *J Clin Endocrinol Metab* 87:4186-4192.
- Damm P, Kuhl C, Bertelsen A, Molsted-Pedersen L. 1992. Predictive factors for the development of diabetes in women with previous gestational diabetes mellitus. *Am J Obstet Gynecol* 167:607-616.
- Darko DA, Dornhorst A, Kennedy G, Mandeno RC, Seed M. 2001. Glycaemic control and plasma lipoproteins in menopausal women with type 2 diabetes treated with oral and transdermal combined hormone replacement therapy. *Diabetes Res Clin Pract* 54:157-164.
- Demerath EW, Towne B, Wisemandle W, Blangero J, Chumlea WC, Siervogel RM. 1999. Serum leptin concentration, body composition, and gonadal hormones during puberty. *Int J Obes Relat Metab Disord* 23:678-685.
- Despres JP, Couillard C, Gagnon J, Bergeron J, Leon AS, Rao DC, Skinner JS, Wilmore JH, Bouchard C. 2000. Race, visceral adipose tissue, plasma lipids, and lipoprotein lipase activity in men and women: The Health, Risk Factors, Exercise Training, and Genetics (HERITAGE) family study. *Arterioscler Thromb Vasc Biol* 20:1932-1938.
- Duncan AC, Lyall H, Roberts RN, Petrie JR, Perera MJ, Monaghan S, Hart DM, Connell JM, Lumsden MA. 1999. The effect of estradiol and a combined estradiol/progestagen preparation on insulin sensitivity in healthy postmenopausal women. *J Clin Endocrinol Metab* 84:2402-2407.
- Elbers JMH, Giltay EJ, Teerlink T, Scheffer PG, Asscheman H, Seidell JC, Gooren, LJG. 2003. Effects of sex steroids on components of the insulin resistance syndrome in transsexual subjects. *Clin Endocrinol* 58:562-571.
- Elkind-Hirsch KE, Sherman LD, Malinak R. 1993. Hormone replacement therapy alters insulin sensitivity in young women with premature ovarian failure. *J Clin Endocrinol Metab* 76:472-475.
- Farrell PM, Engle MJ, Frantz ID, Goldman AS, Kalkhoff R, Kemnitz JW, Perelman R, Stern JS, Susa JB. 1982. Complications of pregnancy and fetal development. *Diabetes* 31:89-94.
- Freinkel N. 1980. Banting lecture 1980. Of pregnancy and progeny. *Diabetes* 29:1023-1035.
- Friday KE, Dong C, Fontenot RU. 2001. Conjugated equine estrogen improves glycemic control and blood lipoproteins in postmenopausal women with type 2 diabetes. *J Clin Endocrinol Metab* 86:48-52.
- Gabal LL, Goodman-Gruen D, Barrett-Connor E. 1997. The effect of postmenopausal estrogen therapy on the risk of non-insulin-dependent diabetes mellitus. *Am J Public Health* 87:443-445.
- Godfrey KM, Barker DJ. 2000. Fetal nutrition and adult disease. *Am J Clin Nutr* 71:1344S-1352S.
- Godsland IF, Crook D. 1994. Update on the metabolic effects of steroidal contraceptives and their relationship to cardiovascular disease risk. *Am J Obstet Gynecol* 170:1528-1536.
- Godsland IF, Crook D, Simpson R, Proudler T, Felton C, Lees B, Anyaoku V, Devenport M, Wynn V. 1990. The effects of different formulations of oral contraceptive agents on lipid and carbohydrate metabolism. *N Engl J Med* 323:1375-1381.
- Godsland IF, Gangar K, Walton C, Cust MP, Whitehead MI, Wynn V, Stevenson JC. 1993. Insulin resistance, secretion, and elimination in postmenopausal women receiving oral or transdermal hormone replacement therapy. *Metabolism* 42:846-853.
- Godsland IF, Walton C, Felton C, Proudler A, Patel A, Wynn V. 1992. Insulin resistance, secretion, and metabolism in users of oral contraceptives. *J Clin Endocrinol Metab* 74:64-70.
- Goldzieher JW, Chenault CB, de la Pena A, Dozier TS, Kraemer DC. 1978. Comparative studies of the ethynyl estrogens used in oral contraceptives: Effects with and without progestational agents on plasma androstenedione, testosterone, and testosterone binding in humans, baboons, and beagles. *Fertil Steril* 29:388-396.
- Goran MI, Ball GD, Cruz ML. 2003. Obesity and risk of type 2 diabetes and cardiovascular disease in children and adolescents. *J Clin Endocrinol Metab* 88:1417-1427.
- Goran MI, Gower BA. 2001. Longitudinal study on pubertal insulin resistance. *Diabetes* 50:2444-2450.
- Goy RW, Kemnitz JW. 1983. Early, persistent, and delayed effects of virilizing substances delivered transplacentally to female rhesus fetuses. In: Zbinden G, Cuomo V, Racagni G, Weiss B, eds. *Application of Behavioral Pharmacology in Toxicology*. New York: Raven Press. p 303-314.
- Gresl TA, Baum ST, Kemnitz JW. 2000. Glucose regulation in captive *Pongo pygmaeus abeli*, *P.p. pygmaeus* and *P.p. abeli* × *p.p. pygmaeus* Orangutans. *Zoo Biology* 19: 193-208.
- Gresl TA, Colman RJ, Roecker EB, Havighurst TC, Huang Z, Allison DB, Bergman RN, Kemnitz JW. 2001. Dietary restriction and glucose regulation in aging rhesus monkeys: A follow-up report at 8.5 yr. *Am J Physiol Endocrinol Metab* 281:E757-765.
- Haarbo J, Marslew U, Gotfredsen A, Christiansen C. 1991. Postmenopausal hormone replacement therapy prevents central distribution of body fat after menopause. *Metabolism* 40:1323-1326.
- Hales CN, Barker DJ. 1992. Type 2 (non-insulin-dependent) diabetes mellitus: The thrifty phenotype hypothesis. *Diabetologia* 35:595-601.
- Hamilton CL, Ciaccia P. 1978. The course of development of glucose intolerance in the monkey (*Macaca mulatta*). *J Med Primatol* 7:165-173.
- Hansen BC, Bodkin NL. 1986. Heterogeneity of insulin responses: Phases leading to type 2 (non-insulin-dependent) diabetes mellitus in the rhesus monkey. *Diabetologia* 29:713-719.
- Hill JO, Sidney S, Lewis CE, Tolan K, Scherzinger AL, Stamm ER. 1999. Racial differences in amounts of visceral adipose tissue in young adults: The CARDIA (Coronary Artery Risk Development in Young Adults) study. *Am J Clin Nutr* 69:381-387.
- Hoar RM, Monie IW. 1981. Comparative development of specific organ systems. In: Kimmel CA, Buelke-Sam J, eds. *Developmental Toxicology*. New York: Raven Press. p 13-33.
- Hudson JC, Baum ST, Frye DM, Roecker EB, Kemnitz JW. 1996. Age and sex differences in body size and composition during rhesus monkey adulthood. *Aging (Milano)* 8:197-204.
- Hulley S, Grady D, Bush T, Furberg C, Herrington D, Riggs B, Vittinghoff E. 1998. Randomized trial of estrogen plus progestin for secondary prevention of coronary heart disease in postmenopausal women. Heart and Estrogen/progestin Replacement Study (HERS) Research Group. *JAMA* 280:605-613.
- Jovanovic-Peterson L, Crues J, Durak E, Peterson CM. 1993. Magnetic resonance imaging in pregnancies complicated by gestational diabetes predicts infant birthweight ratio and neonatal morbidity. *Am J Perinatol* 10:432-437.
- Kanaya AM, Herrington D, Vittinghoff E, Lin F, Grady D, Bittner V, Cauley JA, Barrett-Connor E. 2003. Glycemic effects of postmenopausal hormone therapy: The Heart and Estrogen/progestin Replace-

- ment Study. A randomized, double-blind, placebo-controlled trial. *Ann Intern Med* 138:1-9.
- Kaplan RC, Heckbert SR, Weiss NS, Wahl PW, Smith NL, Newton KM, Psaty BM. 1998. Postmenopausal estrogens and risk of myocardial infarction in diabetic women. *Diabetes Care* 21:1117-1121.
- Kemnitz JW. 1984. Obesity in macaques: Spontaneous and induced. In: Cornelius CE, Simpson CR, Hendrickx AG, eds. *Advances in Veterinary Science and Comparative Medicine*. New York: Academic Press. p 81-114.
- Kemnitz JW. 1994. Effects of gender and age on body size and composition of rhesus monkeys (*Macaca mulatta*). *Am J Primatol* 33:220.
- Kemnitz JW, Francken GA. 1986. Characteristics of spontaneous obesity in male rhesus monkeys. *Physiol Behav* 38:477-483.
- Kemnitz JW, Gibber JR, Lindsay KA, Eisele SG. 1989a. Effects of ovarian hormones on eating behaviors, body weight, and glucoregulation in rhesus monkeys. *Horm Behav* 23:235-250.
- Kemnitz JW, Goy RW, Flitsch TJ, Lohmiller JJ, Robinson JA. 1989b. Obesity in male and female rhesus monkeys: Fat distribution, glucoregulation, and serum androgen levels. *J Clin Endocrinol Metab* 69:287-293.
- Kemnitz JW, Holston KA, Colman RJ. 1998. Nutrition, aging and reproduction in rhesus monkeys. In: Hansel W, Bray GA, Ryan DH, eds. *Pennington Center Nutrition Series. Part IV. Evolution of Research Methods in Nutrition and Reproduction*. Baton Rouge: Louisiana State University Press. p 180-195.
- Kemnitz JW, Sladky KK, Flitsch TJ, Pomerantz SM, Goy RW. 1988. Androgenic influences on body size and composition of adult rhesus monkeys. *Am J Physiol* 255:E857-E864.
- Kim C, Seidel KW, Begier EA, Kwok YS. 2001. Diabetes and depot medroxyprogesterone contraception in Navajo women. *Arch Intern Med* 161:1766-1771.
- Kim C, Siscovick DS, Sidney S, Lewis CE, Kiefe CI, Koepsell TD. 2002. Oral contraceptive use and association with glucose, insulin, and diabetes in young adult women: The CARDIA Study. *Coronary Artery Risk Development in Young Adults*. *Diabetes Care* 25:1027-1032.
- Lee CC, Kasa-Vubu JZ, Supiano MA. 2003. Differential effects of raloxifene and estrogen on insulin sensitivity in postmenopausal women. *J Am Geriatr Soc* 51:683-688.
- Lemieux S, Prud'homme D, Bouchard C, Tremblay A, Despres JP. 1993. Sex differences in the relation of visceral adipose tissue accumulation to total body fatness. *Am J Clin Nutr* 58:463-467.
- Lewis DS, Bertrand HA, Masoro EJ, McGill HC Jr, Carey KD, McMahan CA. 1984. Effect of interaction of gender and energy intake on lean body mass and fat mass gain in infant baboons. *J Nutr* 114:2021-2026.
- Li C, Johnson MS, Goran MI. 2001. Effects of low birth weight on insulin resistance syndrome in Caucasian and African-American children. *Diabetes Care* 24:2035-2042.
- Liew DF, Ng CS, Yong YM, Ratnam SS. 1985. Long-term effects of Depo-Provera on carbohydrate and lipid metabolism. *Contraception* 31:51-64.
- Lindheim SR, Presser SC, Ditkoff EC, Vijod MA, Stanczyk FZ, Lobo RA. 1993. A possible bimodal effect of estrogen on insulin sensitivity in postmenopausal women and the attenuating effect of added progestin. *Fertil Steril* 60:664-667.
- Lobo RA, Bush T, Carr BR, Pickar JH. 2001. Effects of lower doses of conjugated equine estrogens and medroxyprogesterone acetate on plasma lipids and lipoproteins, coagulation factors, and carbohydrate metabolism. *Fertil Steril* 76:13-24.
- Lobo RA, Pickar JH, Wild RA, Walsh B, Hirvonen E. 1994. Metabolic impact of adding medroxyprogesterone acetate to conjugated estrogen therapy in postmenopausal women. The Menopause Study Group. *Obstet Gynecol* 84:987-995.
- Lokkegaard E, Pedersen AT, Heitmann BL, Jovanovic Z, Keiding N, Hundrup YA, Obel EB, Ottesen B. 2003. Relation between hormone replacement therapy and ischaemic heart disease in women: Prospective observational study. *BMJ* 326:426.
- Ludicke F, Gaspard UJ, Demeyer F, Scheen A, Lefebvre P. 2002. Randomized controlled study of the influence of two low estrogen dose oral contraceptives containing gestodene or desogestrel on carbohydrate metabolism. *Contraception* 66:411-415.
- Manning PJ, Allum A, Jones S, Sutherland WH, Williams SM. 2001. The effect of hormone replacement therapy on cardiovascular risk factors in type 2 diabetes: A randomized controlled trial. *Arch Intern Med* 161:1772-1776.
- Manson JE, Rimm EB, Colditz GA, Willett WC, Nathan DM, Arky RA, Rosner B, Hennekens CH, Speizer FE, Stampfer MJ. 1992. A prospective study of postmenopausal estrogen therapy and subsequent incidence of non-insulin-dependent diabetes mellitus. *Ann Epidemiol* 2:665-673.
- Mattiasson I, Rendell M, Tornquist C, Jeppsson S, Hulthen UL. 2002. Effects of estrogen replacement therapy on abdominal fat compartments as related to glucose and lipid metabolism in early postmenopausal women. *Horm Metab Res* 34:583-588.
- McCance DR, Pettitt DJ, Hanson RL, Jacobsson LT, Knowler WC, Bennett PH. 1994. Birth weight and non-insulin dependent diabetes: Thrifty genotype, thrifty phenotype, or surviving small baby genotype? *BMJ* 308:942-945.
- Muller DC, Elahi D, Tobin JD, Andres R. 1996. The effect of age on insulin resistance and secretion: A review. *Semin Nephrol* 16:289-298.
- NAMS [North American Menopause Society]. 2003. Role of progestogen in hormone therapy for postmenopausal women: Position statement of The North American Menopause Society. *Menopause* 10:113-132.
- Oh JY, Barrett-Connor E, Wedick NM, Wingard DL. 2002. Endogenous sex hormones and the development of type 2 diabetes in older men and women: The Rancho Bernardo study. *Diabetes Care* 25:55-60.
- Poehlman ET, Toth MJ, Gardner AW. 1995. Changes in energy balance and body composition at menopause: A controlled longitudinal study. *Ann Intern Med* 123:673-675.
- Polderman KH, Gooren LJJ, Asscheman H, Bakker A, Heine RJ. 1994. Induction of insulin resistance by androgens and estrogens. *J Clin Endocrinol Metab* 79:265-271.
- Pond CM, Mattacks CA. 1987. The anatomy of adipose tissue in captive *Macaca* monkeys and its implications for human biology. *Folia Primatol (Basel)* 48:164-185.
- Ramsey JJ, Laatsch JL, Kemnitz JW. 2000. Age and gender differences in body composition, energy expenditure, and glucoregulation of adult rhesus monkeys. *J Med Primatol* 29:11-19.
- Raudaskoski T, Tomas C, Laatikainen T. 1999. Insulin sensitivity during postmenopausal hormone replacement with transdermal estradiol and intrauterine levonorgestrel. *Acta Obstet Gynecol Scand* 78:540-545.
- Resko JA, Ellinwood WE, Pasztor LM, Huhl AE. 1980. Sex steroids in the umbilical circulation of fetal rhesus monkeys from the time of gonadal differentiation. *J Clin Endocrinol Metab* 50:900-905.
- Reubinoff BE, Wurtman J, Rojansky N, Adler D, Stein P, Schenker JG, Brzezinski A. 1995. Effects of hormone replacement therapy on weight, body composition, fat distribution, and food intake in early postmenopausal women: A prospective study. *Fertil Steril* 64:963-968.
- Rimm EB, Manson JE, Stampfer MJ, Colditz GA, Willett WC, Rosner B, Hennekens CH, Speizer FE. 1992. Oral contraceptive use and the risk of type 2 (non-insulin-dependent) diabetes mellitus in a large prospective study of women. *Diabetologia* 35:967-972.
- Rossouw JE, Anderson GL, Prentice RL, LaCroix AZ, Kooperberg C, Stefanick ML, Jackson RD, Beresford SA, Howard BV, Johnson KC, Kotchen JM, Ockene J. 2002. Risks and benefits of estrogen plus progestin in healthy postmenopausal women: Principal results From the Women's Health Initiative randomized controlled trial. *Jama* 288:321-333.
- Ryan AS, Nicklas BJ, Berman DM. 2002. Hormone replacement therapy, insulin sensitivity, and abdominal obesity in postmenopausal women. *Diabetes Care* 25:127-133.
- Saad RJ, Keenan BS, Danadian K, Lewy VD, Arslanian SA. 2001. Dihydrotestosterone treatment in adolescents with delayed puberty: Does it explain insulin resistance of puberty? *J Clin Endocrinol Metab* 86:4881-4886.
- Saglam K, Polat Z, Yilmaz MI, Gulec M, Akinci SB. 2002. Effects of

- postmenopausal hormone replacement therapy on insulin resistance. *Endocrine* 18:211-214.
- Schwartz SM, Kemnitz JW, Howard CF, Jr. 1993. Obesity in free-ranging rhesus macaques. *Int J Obes Relat Metab Disord* 17:1-9.
- Shadoan MK, Anthony MS, Rankin SE, Clarkson TB, Wagner JD. 2003. Effects of tibolone and conjugated equine estrogens with or without medroxyprogesterone acetate on body composition and fasting carbohydrate measures in surgically postmenopausal monkeys. *Metabolism* 52:1085-1091.
- Shapiro C, Sutija VG, Bush J. 2000. Effect of maternal weight gain on infant birth weight. *J Perinat Med* 28:428-431.
- Simon D, Charles MA, Lahlou N, Nahoul K, Oppert JM, Gouault-Heilmann M, Lemort N, Thibult N, Joubert E, Balkau B, Eschwege E. 2001. Androgen therapy improves insulin sensitivity and decreases leptin level in healthy adult men with low plasma total testosterone: A 3-month randomized placebo-controlled trial. *Diabetes Care* 24:2149-2151.
- Snehaltha C, Ramachandran A, Satyavani K, Vallabi MY, Viswanathan V. 1997. Computed axial tomographic scan measurement of abdominal fat distribution and its correlation with anthropometry and insulin secretion in healthy Asian Indians. *Metabolism* 46:1220-1224.
- Soranna L, Cucinelli F, Perri C, Muzj G, Giuliani M, Villa P, Lanzone A. 2002. Individual effect of E2 and dydrogesterone on insulin sensitivity in post-menopausal women. *J Endocrinol Invest* 25:547-550.
- Southgate DAT, Hey EN. 1976. Chemical and biochemical development of the human fetus. In: Roberts DF, Thomson AM, eds. *The Biology of Human Fetal Growth*. London: Taylor and Francis. p 195-209.
- Spencer CP, Godsland IF, Cooper AJ, Ross D, Whitehead MI, Stevenson JC. 2000. Effects of oral and transdermal 17beta-estradiol with cyclical oral norethindrone acetate on insulin sensitivity, secretion, and elimination in postmenopausal women. *Metabolism* 49:742-747.
- Stevenson JC, Crook D, Godsland IF, Lees B, Whitehead MI. 1993. Oral versus transdermal hormone replacement therapy. *Int J Fertil Menopausal Stud* 38 Suppl 1:30-35.
- Sumino H, Ichikawa S, Itoh H, Utsugi T, Ohyama Y, Umeda M, Nakamura T, Kanda T, Mizunuma H, Tomono S, Murakami M, Kurabayashi M. 2003. Hormone replacement therapy decreases insulin resistance and lipid metabolism in Japanese postmenopausal women with impaired and normal glucose tolerance. *Horm Res* 60:134-142.
- Sumner AE, Farmer NM, Tulloch-Reid MK, Sebring NG, Yanovski JA, Reynolds JC, Boston RC, Premkumar A. 2002. Sex differences in visceral adipose tissue volume among African Americans. *Am J Clin Nutr* 76:975-979.
- Susa JB, McCormick KL, Widness JA, Singer DB, Oh W, Adamsons K, Schwartz R. 1979. Chronic hyperinsulinemia in the fetal rhesus monkey: Effects on fetal growth and composition. *Diabetes* 28:1058-1063.
- Tripathy D, Shah P, Lakshmy R, Reddy KS. 1998. Effect of testosterone replacement on whole body glucose utilisation and other cardiovascular risk factors in males with idiopathic hypogonadotropic hypogonadism. *Horm Metab Res* 30:642-645.
- Triusu RJ, Cowie CC, Harris MI. 2000. Hormone replacement therapy and glucose metabolism. *Obstet Gynecol* 96:665-670.
- Tyagi A, Rajalakshmi M, Jeyaraj DA, Sharma RS, Bajaj JS. 1999. Effects of long-term administration of testosterone enanthate on glucose metabolism in rhesus monkeys. *Contraception* 59:333-337.
- Valdes CT, Elkind-Hirsch KE. 1991. Intravenous glucose tolerance test-derived insulin sensitivity changes during the menstrual cycle. *J Clin Endocrinol Metab* 72:642-646.
- Vela P, Yen SS. 1969. Serum insulin and growth hormone responses to arginine infusion before and during treatment with contraceptive steroids. *J Clin Endocrinol* 29:1212-1216.
- Wagenknecht LE, Langefeld CD, Scherzinger AL, Norris JM, Haffner SM, Saad MF, Bergman RN. 2003. Insulin sensitivity, insulin secretion, and abdominal fat: The Insulin Resistance Atherosclerosis Study (IRAS) Family Study. *Diabetes* 52:2490-2496.
- Wagner JD, Martino MA, Jayo MJ, Anthony MS, Clarkson TB, Cefalu WT. 1996. The effects of hormone replacement therapy on carbohydrate metabolism and cardiovascular risk factors in surgically postmenopausal cynomolgus monkeys. *Metabolism* 45:1254-1262.
- Wagner JD, Thomas MJ, Williams JK, Zhang L, Greaves KA, Cefalu WT. 1998. Insulin sensitivity and cardiovascular risk factors in ovariectomized monkeys with estradiol alone or combined with norethindrone acetate. *J Clin Endocrinol Metab* 83:896-901.
- Wickman S, Saukkonen T, Dunkel L. 2002. The role of sex steroids in the regulation of insulin sensitivity and serum lipid concentrations during male puberty: A prospective study with a P450-aromatase inhibitor. *Eur J Endocrinol* 146:339-346.
- Winborn WB, Sheridan PJ, McGill HC. 1983. Estrogen receptors in the islets of Langerhans of baboons. *Cell Tissue Res* 230:219-223.
- Winborn WB, Sheridan PJ, McGill HC, Jr. 1987a. Localization of progesterin receptors in the islets of Langerhans. *Pancreas* 2:289-294.
- Winborn WB, Sheridan PJ, McGill HC, Jr. 1987b. Sex steroid receptors in the stomach, liver, pancreas, and gastrointestinal tract of the baboon. *Gastroenterology* 92:23-32.
- Writing Group for the PEPI Trial. 1995. Effects of estrogen or estrogen/progestin regimens on heart disease risk factors in postmenopausal women: The Postmenopausal Estrogen/Progestin Interventions (PEPI) Trial. *JAMA* 273:199-208.
- Zhang Y, Howard BV, Cowan LD, Yeh J, Schaefer CF, Wild RA, Wang W, Lee ET. 2002. The effect of estrogen use on levels of glucose and insulin and the risk of type 2 diabetes in American Indian postmenopausal women: The Strong Heart Study. *Diabetes Care* 25:500-504.