The hypothalamus–pituitary–ovary axis and type 1 diabetes mellitus: a mini review

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A high prevalence of menstrual cycle and fertility disturbances has long been associated with diabetes mellitus. However, rationalization of the intrinsic mechanisms of these alterations is controversial and even contradictory. This review considers (i) the relationship between diabetes mellitus, especially type 1 diabetes mellitus (T1DM), and the hypothalamus–pituitary–ovary (HPO) axis, (ii) the state of our knowledge concerning neuroendocrine control and its relationship with dopaminergic and opioid tonus, and (iii) the influence of the hypothalamus–pituitary–adrenal axis on ovarian function. Functional disturbances that occur as a consequence of diabetes are also discussed, but some T1DM-related diseases of autoimmune origin, such as oophoritis, are not further analysed. Although there are clear indications of a relationship between menstrual and fertility alterations and glycaemic control, in many instances the improvement of the latter is not sufficient to reverse such alterations. It appears that the oligoamenorrhoea and amenorrhoea associated with T1DM is mainly of hypothalamic origin (i.e. failure of the GnRH pulse generator) and may be reversible. The importance of the evaluation of the HPO axis in T1DM women with menstrual irregularities, even in the presence of adequate metabolic control, is emphasized.

Key words: Diabetes/fertility/hyperandrogenism/menarche/menstrual dysfunction

Introduction

Systematic studies of the metabolic effects of type 1 diabetes mellitus (T1DM) and type 2 diabetes mellitus (T2DM) on the hypothalamus–pituitary–ovary (HPO) axis have revealed a relationship between these diseases and menstrual disturbances, such as delayed menarche and menstrual cycle irregularities, i.e. amenorrhoea, oligomenorrhoea and polycystic ovary syndrome (Yeshaya et al., 1995, Dorman et al., 2001; Durando et al., 2003). Variations in insulin sensitivity, which are normally observed during the menstrual cycle, are typically exacerbated in patients with menstrual irregularities. These abnormalities may be considered as a possible indicator for predicting the risk of glucose intolerance or of the development of T2DM (Pulido and Salazar, 1999; Cooper et al., 2000; Solomon et al., 2001) within the general population and especially in high-risk groups such as the Pima Indian women of Arizona (Roumain et al., 1998).

The present review focuses primarily on the alterations observed in the HPO axis of T1DM patients.

General principles of the function of the HPO axis

The hypothalamus plays a central role in the hormonal regulation of the female reproductive system. The sequence of events corresponding to the menstrual cycle is induced by the action of hormones released by the hypothalamus–pituitary system on the ovarian follicle (Carr, 1998). The main regulatory factor of reproductive function is GnRH, a decapptide secreted by the ventral medial nucleus of the hypothalamus. Production and further release of GnRH to the portal pituitary system are induced and controlled through stimuli received from other regions of the brain via mediators of different origins.
Amino acids
Glutamate and γ-aminobutyric acid (GABA) are considered, with other neurotransmitters, to be important synaptic regulators, exerting their functions through secretion by hypothalamic nuclei on post-synaptic specific receptors, leading to stimulation (glutamate) or inhibition (GABA) of GnRH neurons (Reichlin, 1998).

Biogenic amines
Monoaminergic substances acting on the hypothalamic region, such as dopamine and serotonin, are inhibitors of GnRH release, while epinephrine and norepinephrine are inducers of release. It is important to note, however, the relative unspecificity of action of these substances, depending on the region considered, receptor characteristics and the influence of other substances. Dopamine, for instance, has both α and β adrenergic stimulation effects, and is converted to norepinephrine in the hypothalamus. Dopamine and norepinephrine alter serotonin secretion, which adds to the complexity of interpretation of their actions on the HPO axis. The regulation of most hypothalamic hormones and peptides is modulated by catecholamines (a subgroup of amines that include dopamine and norepinephrine). Release of GnRH is stimulated by α-adrenergic substances (e.g. ephedrine) and blocked by β-blockers (e.g. phenotolamine), knowledge that provides important tools for experimental and therapeutic purposes (Reichlin, 1998).

Hormones
Prolactin has several effects on gonadotrophin secretion, mainly inhibiting LH and FSH secretion at the pituitary level. Estradiol has a diaphasic effect on the mature pituitary and on hypothalamic GnRH neurons, firstly inhibiting and secondly stimulating its release. LH and FSH exert negative short loop feedback control of GnRH secretion and negative ultrashort loop feedback control of its own secretion (autocrine feedback). Progesterone also has inhibitory direct effects on GnRH neurons. Inhibin, an inhibitory regulator produced by the gonads, and activin, a specific FSH releaser, with follistatin (which inhibits the binding of activin to its receptor) are major regulators of FSH action in women (Reichlin, 1998; Rosenfield, 2002).

Opiod system
Endogenous opium-like peptides, or endorphins, comprise several substances (met-enkephalin, leu-enkephalin, melanocortin, β-endorphin) derived from pro-opiomelanocortin (POMC; a precursor); they inhibit the release of GnRH. Antagonistic action of naloxone usually increases LH and FSH secretion. There is strong evidence that gonadotrophin secretion is regulated by interaction between dopamine and endogenous opioids, although it is not clear if this regulation is carried out directly or is mediated via the dopaminergic system (Djursing, 1987; Reichlin, 1998).

Peptides
This class of organic substances comprises most of the protein compounds with hormonal activity at the hypothalamic and pituitary level. It includes corticotrophin-releasing hormone (CRH), thyrotropin-releasing hormone (TRH), thyroid-stimulating hormone (TSH), vasopressin, oxytocin, somatostatin, neuropeptide Y, melanocortin, growth hormone-releasing hormone growth hormone-releasing hormone (GHRH) and GnRH itself, among about 50 neuropeptides already described and linked to specific neuronal tracts, which exert a wide range of homeostatic and metabolic functions in the HPO axis and other areas of the central nervous system (Reichlin, 1998).

Fernandez-Fernandez et al. (2005) have recently observed in rats a range of effects of gastrointestinal peptide hormones (such as PYY, which acts through the neuropeptide Y central receptors Y1–Y3), ranging from stimulation, inhibition and apparent modulation of LH and GnRH responses depending in part if in vivo or in vitro experiments are considered. This suggests that there is a complex network involving the local hormonal milieu and interaction with other factors, such as leptin, produced by adipose tissue. Vasoactive intestinal peptide seems very important during normal maturation of the HPO axis in rats (Kriegsfeld et al., 2000; Lebrethon et al., 2000) and pigs (Bogacka et al., 2002). These studies reinforce the idea that gastrointestinal peptides, mainly by influencing neuropeptide Y receptors associated with the action of leptin, establishes the hormonal basis for the interaction between energy balance control and reproductive function in mammals.

GnRH, LH/FSH and estrogen physiology
GnRH regulates the synthesis, storage and mobilization of the gonadotrophins as well as their acute release (Carr, 1998). The mechanism of action of GnRH involves the binding of the hormone to a transmembrane receptor, which leads to an increase in cAMP and the subsequent elevation of cytoplasmic Ca2+ or activation of protein kinase C (Conn, 1986; Conn and Crowley, 1991). GnRH, which has a half life time of 2–4 min, reaches the pituitary very rapidly and induces the release of LH and FSH. Normally, GnRH is released in pulse mode at regular intervals of 1 h in order to induce the physiological release of FSH and LH in a pulsating manner. The release of GnRH in continuous mode, or in pulses at intervals longer or shorter than 1 h, does not produce normal pulses of LH and FSH (Carr, 1998). However, in women with a single deficiency of GnRH, pulsed treatment with GnRH was efficient in inducing ovulation and fertility (Knobil, 1980).

There are three distinct patterns of secretion of the gonadotrophins: (i) monthly, characterized by low-level fluctuations in LH and FSH secretion that occur every 30 days during the normal menstrual cycle; (ii) daily, characterized by intermittent LH and FSH release, with larger bursts during sleep. Differences between wake and sleep secretion are larger in girls at the beginning of puberty, with low levels during the day, and increased, progressively higher bursts during sleep. (iii) Hourly, characterized by the pulsed release of LH and FSH (Leyedecker et al., 1980; Reichlin, 1998).

FSH and LH are secreted in a coordinated manner in order to promote the development of the ovarian follicle, ovulation and maintenance of the corpus luteum. The release of FSH and LH is either positively or negatively modulated by estrogen
and progesterone, depending on the concentration and period of exposure of the pituitary to these steroids (Fink, 1988). There is a negative feedback between the secretion of estrogens and the release of gonadotrophins from the hypothalamus (GnRH) and the pituitary (FSH, LH), and this can be particularly noticeable when there is an increase in the secretion of FSH and LH, as in menopausal women and castrated men. The inhibition of FSH and LH may occur when estrogen levels are low, but is more evident when the levels are high. A rapid increase in the level of estrogens can exert a positive effect on the secretion of gonadotrophins, and this is of crucial importance to the production of the LH peak required for the induction of ovulation. Two aspects are important in this mechanism, namely, an estrogen concentration higher than 700 pmol/l (200 pg/ml) and a constant elevated level of estradiol for a period of 48–50 h. Progesterone at high concentrations inhibits the release of both FSH and LH (Carr, 1998) and is also responsible for the FSH peak. At low levels, progesterone stimulates the secretion of LH, but only after lengthy exposure of the pituitary to estrogen.

Together with steroids, gonadal proteins modulate the release of FSH; these proteins comprise activin (which stimulates biosynthesis and release of FSH) and inhibin and follistatin (which specifically suppress the release of FSH; Ying, 1988). The secretion of activin by the granulosa cells of developing follicles increases the release of FSH (Carr, 1998).

Since the mid 1970s, the HPO axis has been evaluated using the GnRH stimulation test, which assesses indirectly the response of the pituitary gland to stimulation by exogenous GnRH. This test permits identification of the causes of gonadal failure, such as different types of amenorrhoea and hypogonadism (Czygan et al., 1974; Donald and Espiner, 1974; Valcke and Mahoudeau, 1974; Keller et al., 1975; Wierdis et al., 1978). Research carried out in Italy involving patients presenting amenorrhoea and eumenorrhoea of diverse aetologies, including diabetes, concluded that the GnRH stimulation test helped with the differential diagnosis of the amenorrhoeas, as well as with the evaluation of pituitary reserves (Wierdis et al., 1978). Comparing the performance of the test described in different reports, however, presents some difficulty since the dose of GnRH typically used to produce an acute stimulus varies between 10 and 100 μg.

**Epidemiology of menstrual disturbances in patients with type 1 diabetes mellitus**

Since the 1950s it has been well recognized that diabetic patients may suffer a delay in their sexual maturity and in the age of menarche, particularly when the onset of diabetes occurred in the pre-pubertal period (Bergquist, 1954; Worm, 1955; Kjaer et al., 1992). Pre-pubertal diabetic children present normal levels of basal gonadotrophins but reduced responses to GnRH, indicating that they probably have limited capacity to maintain an adequate pituitary reserve (Cicognani et al., 1978). In girls presenting the first symptoms of diabetes after the age of 11 (i.e. near to the expected age of menarche), the onset of menarche was much later than in non-diabetic girls, suggesting a disorder of the HPO axis (Schirock et al., 1984). Furthermore, diabetes is also associated with the premature cessation of menstrual cycles, thus leading to a shortening of the fertile period of diabetic patients by up to 17% (Bergquist, 1954; Dorman et al., 2001; Durando et al., 2003). The basic causes of this curtailment of reproductive life may be explained by disorders of the HPO axis, generated by metabolic defects or by adrenal insufficiency, defective opioid modulation of the hypothalamus and autoimmune factors (Snajderová et al., 1999).

There is evidence of a positive correlation between the control of glycaemia and the menstrual cycle in diabetic women. Irregularities, most frequently amenorrhoea and oligomenorrhoea (Djursing et al., 1981), are expected only in the first 2 years following menarche in non-diabetic adolescents (Rosenfield, 2002), but are present in about 20–30% of diabetic patients (Bergquist, 1954; Worm, 1955; Karimova, 1983; Kjaer et al., 1992; Adcock et al., 1994; Yeshaya et al., 1995; Schroeder et al., 2000; Durando et al., 2003; Strotmeyer et al., 2003). The onset of diabetes appears to be an important precipitating factor for the initiation of menstrual disorders, an association that can be clearly observed in at least 50% of these patients (Djursing et al., 1982). The majority of amenorrhoeic diabetic patients present functional amenorrhoea with no evidence of abnormalities in the HPO axis (Worm, 1955; Djursing et al., 1982). Whilst amenorrhoea in non-diabetic patients is provoked by loss of weight (50% of cases) and other unknown factors (Djursing, 1987), in diabetic patients it is triggered by the disease itself (40% of cases) regardless of the cause (i.e. primary, functional or non-specific). One of the specific causes of amenorrhoea may be related to the incidence of autoimmune disorders that exist in T1DM patients. Snajderová et al. (1999) reported a higher prevalence (67.9%) of autoantibodies directed against at least one of the ovarian tissues analysed (ooplasm, pellucid zone, granulosa membrane, internal theca and lutein cells) of juvenile and adolescent diabetic females, when compared with non-diabetic adolescents and young women (4.8%).

Whilst a positive reaction for autoantibodies in the estrogen-producing regions (granulosa and lutein cells) was detected in the same proportions of diabetic patients with and without menstrual irregularities, autoimmune thyroiditis was found in a much larger fraction (80%) of patients with menstrual irregularities.

A study conducted in Russia (Karimova, 1983) involving 157 diabetic women of fertile age (59% of whom had been suffering from the disease for up to 5 years), showed that 73.6% presented obstetric problems and 33% suffered from disturbances of the menstrual cycle. This study indicated a correlation between ovarian dysfunction and severe diabetes.

Another study (Burkart et al., 1989), involving 337 German women with T1DM, concluded that the age of menarche was inversely proportional to the age at which the disease emerged. In patients in whom the disease appeared before puberty, particularly between the ages of 3 and 8 years, menarche occurred 0.8–2 years later than in patients in whom diabetes emerged after menarche and 0.4–1.3 years later than in the healthy population. Furthermore, women suffering from diabetes from an early age presented a more pronounced form of the disease. Primary amenorrhoea appeared in 3.6% of women who had been suffering from diabetes prior to puberty, i.e. younger than
from those of the normal population. Some 14% of the pre-pubertal diabetic group presented oligomenorrhoea and 7% secondary amenorrhoea, whilst the post-pubertal diabetic group showed only secondary amenorrhoea (12%). Irregularities in the menstrual cycle occurred more frequently at the emergence of diabetes and tended to normalize during its evolution. Amongst patients who were 35 years old and above, approximately 70.5% had spontaneous pregnancies and 2.1% were sterile; these percentages were not significantly different from those of the normal population.

A similar study in Denmark (Kjaer et al., 1992) involving 245 diabetic patients and 253 normal women reported that menarche in the pre-pubertal diabetic group occurred 1 year later than in the normal group. In addition, 21.6% of the diabetic patients presented menstrual disturbances compared with only 10.8% \( (P < 0.005) \) of the normal population; thus, 4.9% of the diabetic group presented primary amenorrhoea compared with 1.2% of the normal group \( (P < 0.05) \), 8.2% presented secondary amenorrhoea (cf. normal value of 2.8%; \( P < 0.01 \)), 10.6% presented oligomenorrhoea (cf. normal value of 4.8%; \( P < 0.02 \)) and 7.3% presented polymenorrhoea (cf. normal value of 5.2%; not significantly different).

A retrospective analysis of the regularity of the menstrual cycle, gynaecological history and fertility in 100 diabetic patients in Israel (Yeshaya et al., 1995) showed that the average age of menarche was 13.5 years, with a tendency to be even later in patients with an early history of diabetes, particularly when diagnosed before the age of 10 years.

Schroeder et al. (2000) found a positive correlation between a low level of glycosylated haemoglobin and the regularity of the menstrual cycle in a group of 46 diabetic girls aged between 10 and 18 years. A comparative study (Strotmeyer et al., 2003) carried out in the USA, involving three groups of women (146 with T1DM, 156 of their non-diabetic sisters and 158 unrelated and non-diabetic women) demonstrated that diabetic women had a delayed menarche and a precocious menopause. The prevalence of menstrual disturbances in diabetic women younger than 30 years was higher than in their non-diabetic sisters, although the latter group used more contraceptives; these offer protection against such irregularities. Interestingly, however, the frequency of pregnancies in the diabetic group was lower than in the other groups.

**Diabetes and hypothalamus–pituitary function**

Before the introduction of insulin therapy the incidence of infertility and hypogonadism in diabetic women was greater than 90% (Gilbert and Dunlop, 1949). Whilst insulin treatment can restore fertility in most diabetic women (Skipper, 1933; Bergquist, 1954; Worm, 1955), such patients often suffer menstrual irregularities and a shorter fertile life compared with normal women (Dorman et al., 2001; Durando et al., 2003).

At the end of the 1980s, Djursing (1987) published an extensive review of accumulated knowledge concerning the HPO axis in diabetic patients presenting no menstrual irregularities and in patients suffering from amenorrhoea. It was concluded that a number of pathophysiological mechanisms interfere either directly or indirectly with the homeostasis of the HPO axis. Much of the information provided in that review was obtained from experiments with animal models in which diabetes had been induced. In such studies, the lack of treatment produced collapse of the HPO axis and secondary gonadal malfunction, but adequate insulin replacement restored normal conditions. The degree of gonadal malfunction observed in the animals was proportional to the degree of metabolic disorder that characterized the diabetes (Djursing et al., 1983).

**Studies in animal models**

In diabetic animals, impaired hypothalamus–pituitary function is characterized by low basal levels of FSH and LH accompanied by a normal or low response to GnRH stimulation (Johnson and Sidman, 1979; Kirchick et al., 1979, 1982; Howland and Zebrowsky, 1980), even though the pool of GnRH is intact and the sensitivity of the target organs to gonadotrophins and sexual hormones is normal (Johnson and Sidman, 1979). Furthermore, when appropriate insulin treatment was applied to diabetic experimental animals (Chiiri et al., 1969; Kirchick et al., 1982), the release of gonadotrophins remained inefficient. Thus, it appears that the main aetiological factors associated with impaired hypothalamus–pituitary function are inadequate release of GnRH (Johnson and Sidman, 1979) and/or a reduction in the sensitivity of the pituitary gland to GnRH (Howland and Zebrowsky, 1980). Aloxane-induced diabetic rats, when stimulated by gonadotrophins during the pre-puberal period, did not ovulate and did not present an LH peak (Kirchick et al., 1982). However, ovulation was more regular in groups of diabetic rats that had been treated with insulin with either a variable dose regimen or subcutaneous insulin implants. In particular, ovulation was more regular in the group treated with the implants, demonstrating that continuous liberation of insulin is more efficient in inducing an LH peak.

**Studies in humans**

In humans, studies conducted under hyperglycaemic–hyperinsulinaemic clamp conditions revealed that various groups of women presented diverse glucose metabolism during the different phases of the menstrual cycle. For instance, one subgroup of women with T1DM presented higher hyperglycaemia and lower sensitivity to insulin during the lutein phase. Furthermore, these women showed an increase in the level of estrogens during the change from the follicular to the lutein phase (Widom et al., 1992). Such hormonal alterations may be related to the hypoinsulinaemic status, which affects either the homeostasis of carbohydrate metabolism in the central nervous system or the binding capacity of the reproductive hormones in the central or peripheral nervous systems. Alternatively, the hormonal alterations could be a consequence of the indirect effect of the metabolites generated during ketoadipic and/or hyperglycaemic conditions (Djursing, 1987).

Distiller et al. (1975) observed that the responses to the GnRH stimulation test applied to 20 women with T1DM were similar in patients suffering from oligomenorrhoea or amenorrhoea and those presenting eumenorrhoea. In addition, there was a significant inverse correlation between glycaemia in the...
fasting state and the LH peak, suggesting an influence of glucose metabolism on pituitary function, even though this defective metabolism could not be the sole cause of menstrual abnormalities.

There is evidence that hyperglycaemia interferes with basal levels of gonadotrophins and with levels following stimulation with either GnRH alone or with GnRH, TRH and arginine simultaneously (Vierhapper et al., 1981). These authors studied six T1DM patients under euglycaemic or hyperglycaemic clamp and observed that in both conditions there was no difference with respect to the FSH, LH, TSH or prolactin responses, even in the absence of insulin infusion. However, the response to growth hormone (GH) was suppressed during the hyperglycaemic clamp, and this effect was attributed to the action of arginine on the hypothalamus, suggesting that the modulating influence of hyperglycaemia on the secretion of GH occurs mainly at the hypothalamic level and not at the pituitary level.

In diabetic patients who have not been appropriately treated, the metabolic stress produced by ketoacidosis activates an adrenal catecholaminergic response, since plasma epinephrine and norepinephrine are elevated in such individuals (Christensen, 1970). Although dopamine (a precursor of epinephrine and norepinephrine) does not normally cross the haematic–encephalic barrier, permeability to this neurotransmitter may be altered in diabetic patients (Lorenzi et al., 1980). Dopamine inhibits the secretion of LH (Hagen et al., 1984) and causes a consequent increase in the levels of other catecholamines in the central and peripheral nervous systems that in turn produces a metabolic disorder in non-treated patients, leading to the abnormal secretion of gonadotrophins (Djursing, 1987).

Normal levels of plasma prolactin (Naeije et al., 1979; Bratush-Marrain et al., 1980), as well as moderately elevated levels (Hansen and Torjesen, 1977), have been reported in ketoacidotic diabetic women. In hyperprolactinaemic patients, normal gonadal function may be suppressed because of the direct inhibitory effect of prolactin on ovarian steroidogenesis (Andersen, 1984) together with inadequate release of GnRH caused by defective feedback between estrogens and gonadotrophins (Djursing et al., 1981) or by a short-loop positive feedback of prolactin on the liberation of dopamine (Djursing et al., 1981. In patients suffering from amenorrhoea, an increase in prolactin and FSH following stimulation with metoclopramide (MTC), a dopaminergic inhibitor, was observed (Djursing et al., 1985a, b) but there were no alterations in LH, suggesting an enhancement in dopaminergic activity in these patients.

Together with functional defects in the hypothalamus and the pituitary gland, disturbances in endorphin (endogenous opioid) tonus, leading directly or indirectly to an increase in dopaminergic tonus and a decrease in the LH pulse, have been identified as possible causes of menstrual dysfunction (Griffin et al., 1994; Morley, 1998).

Diabetes and ovarian function

Studies in animal models

Diabetic experimental animals exhibited a decrease in progesterone production (Tesone et al., 1983) and normal or diminished production of estrogens (Kirchick et al., 1978; Katayama et al., 1984). The alteration in steroidogenesis could be related either to the reduction in GnRH and secretion of gonadotrophins (Johnson and Sidman, 1979) or to a reduction in the affinity or number of gonadotrophin receptors in the ovarian cells (Tesone et al., 1983).

Studies in humans

Ovarian function may be impaired with respect to steroidogenesis as a direct or indirect consequence of the defective HPO axis. The abnormal secretion of steroids was demonstrated by Djursing et al. (1985b) in a study including diabetic and non-diabetic women suffering from amenorrhoea or without amenorrhoea. The diabetic amenorrhoeic group showed low levels of steroid hormone-binding globulin (SHBG), estradiol, testosterone and other androgenic and estrogenic steroids when compared with both the diabetic and the non-diabetic eumenorrhoeic groups. The levels of Δ4-androstenedione and testoster- one were higher in the diabetic eumenorrhoeic group compared with their non-diabetic counterparts. It was concluded that the menstrual abnormalities could be a consequence of the suppression of the HPO axis, since the slight hyperandrogenism revealed by the diabetic amenorrhoeic patients could not explain the relationship between amenorrhoea and diabetes.

The low estrogen levels in diabetic amenorrhoeic women were attributed to ovarian suppression, whilst the high levels of androgens in diabetic eumenorrhoeic women were considered to be of ovarian origin. Glud et al. (1982) also detected a decrease in testosterone and androstenedione levels in six ketotic diabetic women of fertile age, suggesting suppression of the hypothalamus–pituitary system.

Adcock et al. (1994) reported that diabetic women suffering from menstrual irregularities also presented impaired metabolic control, lower levels of SHBG and higher LH/FSH ratios. Furthermore, 77% of these women presented signs of polycystic ovary syndrome.

The evidence concerning steroidogenic dysfunction amongst T1DM women suffering from amenorrhoea is further corroborated by measurements of the estrogen/progesterone ratio. Zumoff et al. (1990) demonstrated that in T1DM patients this ratio was two-fold higher during the follicular phase than that presented by non-diabetic women. It was suggested that these patients were at risk of developing atherogenic and cardiovascular problems, since the elevated estrogen/progesterone ratio was due partly to an increase in serum estradiol levels and also to a decrease in progesterone, which is a protective factor against coronary artery disease.

Figure 1 summarizes the physiopathology of diabetes with respect to the HPO axis, and shows the most important features associated with the relationship between diabetic hyperglycaemia and metabolic and hormonal dysfunction, leading to the impairment of gonadal function in women with T1DM.

Evaluation of the HPO axis in patients with type 1 diabetes mellitus and regular menstrual cycles

Alterations in HPO function in T1DM patients with normal menstrual cycles are presumably quite minor since ovulation is
preserved (Bergquist, 1954; Worm, 1955). However, even these small alterations in the HPO axis, although not generating menstrual disturbances, provide an insight into menstrual dysfunction in diabetic patients (Djursing, 1987). For instance, it is known that diabetic women (particularly of perimenopausal age) present slightly reduced levels of prolactin, consistent with a small increase in dopaminergic tonus, together with a diminished response of the lactotrophs to TRH stimulation. The fact that, following administration of MTC, the prolactin levels in diabetic women with normal menstrual cycles are the same as those found in non-diabetic women leads to the conclusion that the lower basal levels and the reduced secretion of prolactin found in the former group are a consequence of the effect of a slight increase in dopaminergic activity on the lactotrophs (Djursing, 1987).

With respect to the gonadotrophins, diabetic patients with normal menstrual cycles present normal levels in the follicular phase (Distiller et al., 1975), and normal pulses (Djursing et al., 1985a), which are not affected by the dopaminergic blockade induced by MTC. The logical conclusion is that the dopaminergic activity is normal or that the GnRH-producing neurons are insensitive to changes in dopaminergic activity (Djursing, 1987).

In diabetic patients with regular menstrual cycles, the fluctuation of plasma catecholamines is modest, showing an increase in peripheral plasma dopamine and a decrease in conjugated dopamine. This relative increase in the ratio between active dopamine and conjugated dopamine could exert an effect on prolactin found in the former group are a consequence of the effect of a slight increase in dopaminergic activity on the lactotroths (Djursing, 1987). With respect to the gonadotrophins, diabetic patients with normal menstrual cycles present normal levels in the follicular phase (Distiller et al., 1975), and normal pulses (Djursing et al., 1985a), which are not affected by the dopaminergic blockade induced by MTC. The logical conclusion is that the dopaminergic activity is normal or that the GnRH-producing neurons are insensitive to changes in dopaminergic activity (Djursing, 1987).

In diabetic patients, the secretion of GH is stimulated by dopamine, the highest levels being detected at the follicular phase of the cycle. MTC blockage, however, does not produce a significant increase in GH levels, the reason for which may be that dopamine influences somatostatin and GHRH. Two hypotheses can be deduced from this situation: (i) In each of the systems there are different affinities that may influence the

Figure 1. Schematic representation of hypothalamus–pituitary–ovary (HPO) axis in normal and diabetic women. The inhibitory effects of diabetic hyperglycaemia on the HPO axis are represented through the inter-relationships of metabolic dysfunction in both opioid and dopaminergic systems, and the hypothalamus–pituitary–adrenal axis. The plausible inhibitory effect of autoantibodies on the ovarian function is also represented. H = hypothalamus; P = pituitary; O = ovary; BBB = blood–brain barrier; DA = dopamine; PRL = prolactin; DKA = diabetic ketoacidosis. Arrows: continuous lines, stimulation; dashed lines, inhibition; interspaced lines, modulation.
effect of MTC on the release of GH; and (ii) MTC has little influence on either system (Djursing et al., 1984). The conclusion is that the augmentation of dopaminergic activity interferes with the modulation of both TSH and GH (Djursing, 1987).

The abnormalities found in eumenorrheic diabetic women can also be explained by a disruption of opioid modulation that progresses with the disease. Coiro et al. (1991) analysed 29 eumenorrheic diabetic women, one group of whom had been suffering from the disease for between 3 and 9 years and the other for 10–20 years. The two groups were subjected to GnRH or naloxone (an opioid antagonist) stimulation: the LH response in the first group was similar to that of normal women but the second group presented a reduced LH response, indicating a negative correlation between the duration of the disease and the LH peak.

Reports concerning the levels of sex steroids in diabetic patients are not completely consistent. Diabetic patients with no menstrual disturbances were found to present normal levels of estrogens and elevated androstenedione and testosterone (Djursing et al., 1985b), but normal levels of testosterone in such patients have also been reported (Glud et al., 1982). This discrepancy may be due to the different criteria used for the selection of patients in these studies (Djursing, 1987). Androstenedione is converted to testosterone and, since a direct correlation between androstenedione and testosterone in T1DM patients with normal cycles has been found, the increased testosterone levels observed may be a consequence of increased secretion of androstenedione (Djursing et al., 1985b). Despite the high levels of androstenedione and total testosterone recorded exclusively in diabetic patients with normal cycles, there was no increase in free testosterone or in dihydrotosterone, the formation of which depends on 5α-reductase activity. These results explain why there were no signs of hyperandrogenism or menstrual dysfunction in these patients despite their high levels of total androgens (Djursing et al., 1985b). Furthermore, the levels of SHBG were increased in such patients; whilst synthesis of this protein is stimulated by estrogens and thyroid hormone, it is inhibited by GH and androgens and by reduced sensitivity of the target organs (Djursing et al., 1985b; Djursing, 1987).

**Evaluation of the HPO and hypothalamus–pituitary–adrenal axes in patients with type 1 diabetes mellitus and functional amenorrhea**

Normally, evaluation of the HPO axis in diabetic patients suffering from functional amenorrhea reveals hypofunction, with disorders of the feedback mechanisms that appear to be independent of the dose of insulin being used and of the age at diagnosis of diabetes. In addition, these disorders are apparently not coupled with the duration of treatment but are predominantly related to the dopaminergic inhibition that is often associated with the dysfunction of opioid peptides, which act as hypothalamic modulators (Djursing, 1987).

In amenorrheic diabetic women, basal FSH levels are low even when basal estrogens are low, but the response of FSH to GnRH stimulation remains normal. This suggests insufficient secretion of gonadotrophins or a disturbance in the feedback mechanism in such patients (Djursing et al., 1983, 1985b).

South et al. (1993) compared LH levels in eumenorrheic non-diabetic women and in women with T1DM who had unsatisfactory metabolic control and secondary amenorrhea. Determination of LH was carried out over a period of 24 h following GnRH stimulation, and the results showed that, compared with the control group, there was a reduction in the number of LH peaks during the day in diabetic women, but that the amplitudes and areas of the peaks were larger. This hyper-response to GnRH suggested that secondary amenorrhea is more connected to dopaminergic tonus (interference in the generation of hypothalamic pulses) than to pituitary malfunction (deficient liberation of gonadotrophins).

Attempts to control the excess dopaminergic activity presented by amenorrheic diabetic women through administration of MTC resulted in a doubling of FSH levels (Hagen et al., 1983) but, in contrast to the situation for amenorrheic non-diabetic women, did not result in increases in estradiol and LH (Djursing et al., 1985a). This suggests a lack of response of the ovary to FSH, leading to disruption of the positive feedback mechanism for LH. The basal levels, both amplitude and pulse number, of LH are diminished in diabetic patients with menstrual disturbances and eumenorrhea compared with non-diabetic patients (Djursing et al., 1985a). The response to GnRH is also compromised and correlates positively with the low levels of estrogens (Djursing et al., 1983). Since the sensitivity of the LH-producing gonadotrophs to GnRH is modulated by estrogens (Djursing et al., 1983) and the capacity of the gonadotrophs to respond to estrogenic feedback is intact, the reduced capacity for the production of estrogen could be one of the reasons why amenorrheic diabetic women produce low levels of LH (Djursing et al., 1985b). Alternatively, the insufficiency of LH could be caused by depletion of GnRH occasioned by its inadequate release, a reduction in the sensitivity of the gonadotrophs to GnRH, or the failure of the pituitary to secrete LH after an extended period of GnRH deprivation.

Dopaminergic activity is probably elevated in amenorrheic non-diabetic women presenting an absence of LH response to the dopaminergic agonist bromocriptine (Djursing et al., 1981) and a positive LH response to the dopaminergic antagonist MTC (Hagen et al., 1983). The administration of MTC induces a significant LH response in a larger proportion of amenorrheic diabetic women compared with eumenorrheic diabetic and non-diabetic women (Djursing et al., 1985a), suggesting greater dopaminergic suppression of LH secretion in the first group (Hagen et al., 1983). This mechanism could be mediated by the increased GnRH that accumulates inside the neurons of the hypothalamus as a result of suppressed secretion (Djursing, 1987). Lifting the dopamine inhibition may result in increases in GnRH and gonadotrophins.

The basal prolactin concentration is diminished in amenorrheic diabetic women compared with eumenorrheic diabetic and non-diabetic women (Djursing et al., 1982). The inhibition of prolactin is mediated by the dopamine receptors present in the lactotrophs; therefore the increase in dopaminergic activity may be the principal cause of the low levels of prolactin found in amenorrheic and even in eumenorrheic diabetic women. In amenorrheic diabetic women the prolactin...
response to the TRH test is normal (Djursing et al., 1983), indicating that the integrity of the receptors for TRH in the lactotrophs is preserved. However, the pool of available prolactin in such patients is lower, as indicated by a reduced prolactin response to MTC-mediated dopaminergic inhibition in comparison with that exhibited by eumenorrhoeic diabetic and normal women (Djursing et al., 1985b). The reduction in the available pool of prolactin following prolonged exposure to excess dopaminergic activity may be a consequence of the down-regulation of the TRH receptors or a decrease in their number or activity. Since estrogens increase the release of prolactin in normal women, the fact that the basal levels of these steroids are diminished in amenorrhoeic diabetic women may contribute to the low levels of prolactin found in these patients (Djursing, 1987).

Although the peripheral levels of non-conjugated dopamine are low and the ratio of free to conjugated dopamine is more elevated in amenorrhoeic diabetic women, the levels of epinephrine and norepinephrine remain unaltered. There is no evidence of a correlation, however, between the levels of peripheral catecholamines and the secretion of the hypothalamus–pituitary hormones that are modulated by dopamine (Djursing, 1987).

The role of endogenous opioids
A review from Morley (1983) stressed the inhibitory effects exerted by opioid compounds on the liberation of GnRH from the hypothalamus. Endogenous opioids appear to have little effect on the basal secretion of prolactin, unlike pharmacological doses of opioid agonists that increase the secretion of prolactin, leading to hypogonadism and impotence in men submitted to intrathecal opioid therapy for the cure of non-tumoral chronic pain (Roberts et al., 2002). Whilst opioid antagonists have no effect on the increase in MTC-induced prolactin (Laurian et al., 1981), dopamine inhibits the increase in gonadotrophins induced by opioid antagonists (Delitala et al., 1980). Based on such evidence, it is believed that gonadotrophin secretion is regulated by an interaction between dopamine and endogenous opioids, although it is not clear if this regulation is carried out directly or is mediated via the dopaminergic system (Djursing, 1987).

O’Hare et al. (1987) studied the response to the opioid inhibitor naloxone in five hypogonadotrophic amenorrhoeic women with T1DM whose disease had not been fully controlled. Following successful intensification of the insulin treatment, the levels of FSH and LH in all patients remained unchanged and, furthermore, menstruation was not initiated. GnRH inhibition mediated by opioid compounds occurs in some amenorrhoeic women (Sauder et al., 1984), suggesting that the alterations in basal and GnRH-stimulated LH found in these patients result, in part, from modification of the activity of endogenous opioids. Corroborating evidence of alterations in plasma opioid activity in diabetic humans has been provided by several reports (Awoke et al., 1984; Caprio et al., 1991) showing that diabetic patients respond well to opioid inhibition therapy with naloxone, resulting in more efficient control of hypoglycaemia. Such positive effects may be explained by the improvement in cortisol and epinephrine release that had been suppressed by endogenous opioid modulation in these patients (Caprio et al., 1991).

Thyroid function
TSH levels are significantly lower in amenorrhoeic diabetic women compared with eumenorrhoeic diabetic and non-diabetic women. The plasma thyroid hormone levels are also normal and there is no association with alterations in rT3 (Djursing et al., 1982). This suggests that the low TSH levels are caused by the reduced secretion of TSH rather than by alterations in the binding capacity or peripheral metabolism of the thyroid hormones. Dopamine inhibits the release of TSH at the hypothalamus–pituitary level and the TSH response to the blockade of dopaminergic receptors by MTC is normal in amenorrhoeic diabetic women. The enhancement in dopamine activity in the TRH-releasing neurons and pituitary thyrotrophs explains the low basal TSH and the normal TSH response following the use of MTC. Because dopamine inhibits the release of TRH, treatment with MTC liberates the pool of this hormone. However, the effect of high TRH is counterbalanced by the inability of the pituitary to respond with the discharge of TSH owing to the chronic inhibition of dopaminergic activity. Basal GH is also increased in amenorrhoeic diabetic women and, because there is a correlation between the secretion of GH and dopaminergic tonus, the elevated basal GH levels suggest that these patients are under the influence of high central dopaminergic activity (Djursing, 1987).

The role of androgens and the hypothalamus–pituitary–adrenal axis

Studies in animal models
Hyperactivation of the hypothalamus–pituitary–adrenal (HPA) axis was observed in diabetic rats, the stress responses of which were defective and involved a diminution of pituitary corticotroph sensitivity to CRH as well as a reduction in adrenal cortex sensitivity to adrenocorticotropic hormone (ACTH) (Chan et al., 2002). Such hyperactivation could be partially repaired by administration of insulin and normalization of pituitary–adrenal function. Thus, hyperactivation of the HPA axis may be seen as a consequence of an increase in central activity or a decrease in the sensitivity of the negative feedback mechanism of glucocorticoids.

Studies in humans
Increased levels of ovarian and adrenal androgens may be the cause of amenorrhoea in women (Carr, 1998). However, studies have demonstrated that amenorrhoeic diabetic women present lower levels of estrogens, androgens and their precursors compared with eumenorrhoeic diabetic women. Furthermore, amenorrhoeic diabetic women exhibit lower levels of SHBG (Anderson, 1974; Djursing, 1987) and less dihydrotosterone and estradiol (both free and protein-bound) compared with normal women (Djursing et al., 1985b). In fact, steroid production in the ovaries is defective in amenorrhoeic diabetic women, and this condition is due in part to inadequate gonadotrophic stimulation, as confirmed by the inferior levels of basal
and GnRH-stimulated LH found in these patients (Djursing et al., 1983, 1985b).

Whilst the diurnal excretion of pregnanetriol was normal in amenorrhoeic diabetic women (Djursing et al., 1982), the levels of dehydroepiandrosterone sulphate were lower than in eumenorrhoeic diabetic and normal women (Djursing et al., 1985b). Some amenorrhoeic diabetic women presented a modest increase in free cortisol excreted in the urine (Djursing et al., 1982), probably because of increased production since there was no renal impairment in these patients. None of the group presented clinical signs of hypercortisolism. Glucocorticoid hyperactivity is generally accompanied by an increase in estrogenic and androgenic activity, but this condition is not normally observed in amenorrhoeic diabetic women. Typically there is no correlation between changes in adrenal hormone parameters and alterations of the gonadotrophic axis (Djursing et al., 1985b), making adrenal hyperactivity an unlikely explanation for the incidence of amenorrhoea amongst diabetic women (Djursing, 1987). Virdis et al. (1997), following studies on a group of oligomenorrhoeic adolescents with T1DM before and after stimulation with an GnRH analogue (leuprolide), suggested that adrenal hyperactivity may precede and cause partial gonadotrophic insufficiency, with progressive disarray of the hypothalamic generator of GnRH pulses. Their conclusions were based on the fact that a higher 17-hydroxyprogesterone response was observed in this group than in eumenorrhoeic diabetic and normal women.

The HPA axis was studied in both amenorrhoeic and eumenorrhoeic diabetic women (De Veo et al., 1999), and the results showed that the former group had lower basal levels of LH, FSH, prolactin, estradiol, androstenedione and 17-hydroxyprogesterone compared with the latter, as well as a diminished ACTH response to the CRH test and a diminished prolactin response to the MTC inhibition test. However, the prolactin response to the CRH test and the 24 h cortisol evaluation test (notably between 0 and 10 a.m.) provided higher values for the amenorrhoeic than for the eumenorrhoeic patients, indicating hyperactivation of the HPA axis. This study emphasized the importance of considering well-controlled amenorrhoeic diabetic patients as having functional amenorrhoea that requires specific clinical treatment. Other studies support the role of the HPA axis in the metabolic effects of diabetes, such as the counter-regulation of hypoglycaemia and disturbances in the response to stress.

Conclusions

For better evaluation of menstrual disturbances in diabetic patients, mainly those presenting amenorrhoea and alterations in the duration of the cycle, it seems worthwhile to include an evaluation of the integrity of the HPA axis, as determined by quantification of prolactin, adrenal hormones, estrogens and androgens. Such data could be very valuable with respect to patients in whom medication produces an improvement in metabolic control but in whom normal menstrual cycles are not re-established. It appears that, in such cases, menstrual disturbances are not related to any particular intensity of metabolic disarray. Furthermore, interfering factors are numerous and the relative influence of the lack of full metabolic control on the generation of such disorders varies individually.

Unfortunately, the therapeutic approaches used to treat menstrual disturbances and to control diabetes are still restricted. The use of dopaminergic inhibitors, such as MTC, together with erythromycin, cisapride and domeridone, is limited to cases of diabetic gastroparesis that do not respond to changes in diet (Smith and Ferris, 2003).

Studies concerning the use of opioid inhibitors, such as naloxone and naltrexone, are limited to acute responses, generally involving intravenous infusions to stimulate the intensity of the opioid barrier (O’Hare et al., 1987). More recent studies suggest the use of opioid inhibitors in regenerative processes; for example, a 4-week application of naltrexone in the treatment of cornea lesions in rats leads to more rapid recovery (Zagon et al., 2002). Moreover, Rainear et al. (2004) treated women with T1DM who presented nutritional psychiatric disorders (bulimia and binge-eating) with naltrexone for 1 year and achieved satisfactory results.

Studies on critically ill patients maintained in an intensive therapy unit revealed that the response of the hypothalamus–pituitary undergoes alteration in two distinct phases, acute and chronic, with detectable effects in all areas (somatotrophic, thyrotrophic, gonadotropic and adrenocorticotropic) (Van Den Berghe, 2002). The initial release of secretagogues (GHRH, TRH, GnRH and ACTH) could be detected, even though there was no effective peripheral response; i.e. only the gonadotropic sector was affected. In the acute phase there was an increase in LH liberation whilst testosterone was reduced, in contrast to the chronic phase, when both LH and testosterone levels were diminished in the serum. In the light of such evidence, consideration has been given to the potential therapeutic use of secretagogues as part of a strategy rapidly to reverse the inhibition of the hypothalamus–pituitary system in critically ill patients, leading to shortening of hospital confinement and improvement in the lifespan of these patients (Weekers and Van Den Berghe, 2004). Furthermore, studies on the hypothalamus–pituitary response in critical patients under chronic stress have led to the belief that similar mechanisms of inhibition might act in amenorrhoeic diabetic women. Therefore, stimulation with secretagogues (i.e. GnRH) might help with the normalization of gonadotrophic function in patients whose metabolic balance has been improved but in whom the re-establishment of normal menstrual cycles has not been accomplished.

Finally, we conclude that T1DM, like diabetes mellitus in general, must be considered in the differential diagnosis of amenorrhoea. Studies carried out to date have not clarified all of the mechanisms involved in this disorder. The only clear evidence is for the augmentation of dopaminergic tonus with the consequent alteration in the menstrual cycle and in GnRH, FSH, LH and estradiol feedback. The hyperandrogenism commonly associated with menstrual irregularities in non-diabetic women cannot be fully explained. Since most studies suggest that there is an individual response to the improvement of glycaemic control, it is worthwhile evaluating the hormonal status of those diabetic patients in whom menstrual irregularities persist even though metabolic control has been improved. There is no conclusive evidence concerning the use of dopaminergic...
inhibitors for the treatment of diabetic patients suffering from menstrual irregularities, although there is confirmation that the use of hypothalamus–pituitary secretagogues may have a normalizing action on the HPO axis, which is chronically suppressed in amenorrheic diabetic patients.

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