Investigating the Response to Metformin in Polycystic Ovary Syndrome With Metabolomics

Polycystic ovary syndrome (PCOS), a condition of androgen excess and chronic anovulation, is the most common endocrine disorder among women of reproductive age. Alice Y. Chang, M.D., of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic’s campus in Rochester, Minnesota, says: “Little is known about the pathophysiology of insulin resistance in PCOS or the exact mechanisms for one of its few therapies, metformin, an insulin sensitizer that can increase ovulatory cycles and lower androgens. There is significant heterogeneity in PCOS — in clinical presentation, diagnostic criteria, and response to metformin and anti-androgen therapies. This heterogeneity confounds current and future research on underlying causes and novel therapies. For women with PCOS, therapeutic options are limited and approached in a systematic though empiric, ‘try and see what works’ approach. Taking advantage of the observations that metformin can have significant effects in some women with PCOS while little effect in others, we are conducting a study to better understand the response to metformin in PCOS and the underlying pathways affected. Because women with PCOS have a higher prevalence of cardiovascular risk factors and a higher risk of developing diabetes, our study will focus on the response to metformin as measured by changes in insulin sensitivity, endothelial function and arterial stiffness.”

Metabolomics: The next frontier of systems biology and personalized medicine
Metabolomics is the study of small molecules that can serve as a fingerprint of underlying
cellular biochemical processes resulting from final expression via transcriptome and proteome. In most cases, the gene expression is influenced by the environment (Figure, page 1).

K. Sreekumaran Nair, M.D., Ph.D., of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic’s campus in Minnesota, is the principal investigator of one of six Regional Comprehensive Metabolomics Research Cores supported by the National Institutes of Health Common Fund program.

Dr. Nair explains: “For a heterogeneous condition such as PCOS, metabolomics may lead to the development of new biomarkers for identifying specific phenotypes within PCOS, such as groups at risk of diabetes or heart disease. Metabolomics can also identify new potential drug targets for pharmacotherapy and those who may respond to pharmacological interventions. In addition, the established Mayo expertise in the measurement of stable isotopes to study fuel flux changes offers opportunities to understand the underlying mechanism of changes in metabolite concentrations. The ultimate goal of the research is to identify the disease at various stages by reliable biomarkers and implement effective therapies.”

**PCOS: An opportunity to prevent diabetes and heart disease in women**

Heart disease is still the leading cause of death in women. Diabetes significantly increases the risk of heart disease in women, virtually eliminating the gender differences favoring women before menopause. Dr. Chang notes: “Given the higher prevalence of prediabetes and diabetes in women with PCOS, PCOS can be an ideal model to better understand how the various factors — genetic, metabolic and environmental — contribute to the development of diabetes and symptomatic heart disease. Despite the increased prevalence of cardiovascular risk factors in women with PCOS, studies have yet to confirm an increase in cardiovascular events later in life. Given the heterogeneity in PCOS, it is likely that we need better ways to define groups at higher cardiovascular risk. A secondary goal of the response to the metformin study will be to evaluate the changes in endothelial function as an important risk factor specifically in women. Women are more likely to develop symptomatic coronary endothelial function before the typical plaque-based coronary artery disease.”

Amir Lerman, M.D., of the Division of Cardiovascular Diseases at Mayo Clinic’s campus in Minnesota, and chair of Cardiovascular Research at Mayo Clinic and director of the Chest Pain and Coronary Physiology Clinic, is an expert in coronary physiology and endothelial function and a collaborator in the PCOS study. Dr. Lerman highlights: “Subclinical measurements of cardiovascular disease that have been validated in other populations, such as endothelial function and carotid intima medial thickness, are generally worse in women with PCOS than in women without PCOS. However, the studies are quite heterogeneous, and some suggest more or less of an influence of body mass index and insulin resistance. We will be evaluating the effect of metformin on endothelial function, using the EndoPAT, a measurement of peripheral arterial tonometry in response to flow-mediated dilation. In addition, we will evaluate the response to metformin through endothelial progenitor cell function and inflammatory markers.”

Dr. Chang concludes: “In the clinical setting, women with PCOS may have a wide range of concerns, depending on their symptoms and whether they are focused on any challenges or potential challenges with fertility. Although metabolomics profiling is in the early research stage toward a systematic approach to personalized medicine, clinical consultations today can still use the best of the current understanding of PCOS and clinically available tests to refine and individualize treatment to the patient. With a thorough exploration — evaluating family history, testing for prediabetes, dyslipidemia and the severity of the PCOS phenotype — women with PCOS can be guided through an individualized assessment and treatment plan.”
The Role of Focused Parathyroidectomy

Primary hyperparathyroidism (HPT) is the most common cause of hypercalcemia, affecting as many as 1 in 750 people and 1 in 500 women over the age of 60 years. Geoffrey B. Thompson, M.D., of the Department of Surgery at Mayo Clinic’s campus in Rochester, Minnesota, says: “For many years, standard bilateral cervical exploration was the gold standard, with a cure rate higher than 98 percent and risk of recurrent laryngeal nerve damage or hypoparathyroidism of less than 1 percent. However, bilateral exploration generally requires a larger incision and involves operative exploration of both sides of the central neck, thereby potentially engendering risk to recurrent laryngeal nerves as well as all four parathyroid glands. With the introduction of several key advances, this highly successful operation has been further refined with patient-focused improvements that have prompted a shift from the standard bilateral surgical approach to a more focused operation.” Focused parathyroidectomy entails an image-guided exploration of a single parathyroid adenoma with the utilization of intraoperative parathyroid hormone (PTH) monitoring to define biochemical cure.

Focused parathyroidectomy has emerged as a popular technique in endocrine surgery (Figures 1-4). Key advances that have facilitated the development of focused parathyroidectomy are threefold:

- High-quality parathyroid adenoma localization techniques
- Highly accurate rapid intraoperative PTH monitoring
- The ability to carry out these operations through smaller incisions, often resulting in earlier hospital dismissal and return to usual activities

High-quality localization techniques
Melanie L. Richards, M.D., of the Department of Surgery at Mayo Clinic’s campus in Minnesota, says: “Considerable improvements in and widespread use of sophisticated and accurate imaging modalities have allowed for preoperative localization of a single parathyroid adenoma in the majority of patients with primary HPT. Preoperative localization allows for the focused exploration and resection of the single offending gland without the need for bilateral four-gland exploration. Frequently utilized imaging modalities include parathyroid technetium-sestamibi (MIBI) scintigraphy, ultrasonography and 4-D computerized tomography (SPECT) and CT fusion has become the localization procedure of choice at Mayo Clinic. It is minimally invasive and depends on physiologic hyperfunction of the enlarged parathyroid gland rather than on purely anatomic identification. Adenomas anywhere in the neck or mediastinum can be localized with the addition of SPECT and CT fusion images. MIBI scanning is somewhat less dependent than cervical ultrasonography on the size of the parathyroid adenoma for imaging, but the cost is usually higher. A major advantage of MIBI scanning over ultrasonography is that it is less dependent on operator experience to obtain a high-quality scan. The sensitivity of MIBI scanning is 75 to 88 percent.”

Travis J. McKenzie, M.D., of the Department of Surgery at Mayo Clinic’s campus in Minnesota, adds: “High-resolution parathyroid ultrasonography has gained popularity in recent years. It is noninvasive and the most inexpensive preoperative localization technique. Unlike parathyroid scintigraphy and CT, there is no radiation exposure to the patient. Ultrasound is anatomically precise and capable of identifying 95 percent of adenomas weighing more than 1,000 mg. However, ultrasonography identifies less than 50 percent of adenomas weighing less than 200 mg. Its sensitivity and specificity are approximately 60 percent and 90 percent, respectively. Ultrasound carries the added advantage of concomitantly identifying thyroid pathology. The key limiting factor for ultrasonographic localization is its dependence on a skilled operator. Furthermore, ectopic glands located in the mediastinum or tracheoesophageal groove are not readily identifiable with ultrasound, and adenomas may be obscured in the setting of nodular thyroid disease.
A newer imaging modality, 4D-CT employs multiphase contrast-enhanced CT imaging relying on the early arterial contrast enhancement and washout to identify parathyroid glands. This technique provides highly accurate anatomic detail that a clinician can readily interpret. In addition, 4D-CT has been demonstrated to be > 90 percent accurate in identifying single adenomas, with an overall sensitivity ranging from 70 to 90 percent. Furthermore, this modality provides excellent anatomic imaging of the tracheoesophageal groove and retroesophageal ectopic glands as well as the mediastinum. However, 4D-CT is costly and results in radiation exposure.

High-quality, rapid intraoperative PTH measurement

David R. Farley, M.D., of the Department of Surgery at Mayo Clinic’s campus in Minnesota, says: “Intraoperative PTH monitoring has a turnaround time of approximately 30 minutes or less and can therefore be utilized in the operating room to confirm resection of the offending parathyroid adenoma by demonstrating a rapid decline in PTH levels. This allows for highly accurate confirmation of surgical cure without the need for four-gland cervical exploration in patients with single adenomas. The accuracy of intraoperative PTH measurement has been widely verified and enthusiastically supported. Cost ranges from $350 to $750 a patient.”

Small-incision outpatient procedure

The option of local anesthesia, a small incision and brief outpatient recovery time are further advantages of the unilateral focused approach.

The Mayo Clinic experience

Dr. Thompson explains: “In June 1998, we introduced focused parathyroidectomy at Mayo Clinic. Through September 2009, we performed 3,203 parathyroid operations. During recent years, 75 percent of patients had the focused procedure. Ninety-seven percent of patients who required a standard four-gland exploration had specific contraindications for the focused approach. The cure rate for both conventional open exploration and focused parathyroidectomy was 97 percent.”

Dr. Richards highlights: “Localization was performed with MIBI scanning and ultrasonography in the majority of cases. The sensitivity and positive predictive value of MIBI were 86 percent and 92 percent, respectively; comparable figures for ultrasonography were 62 percent and 90 percent, respectively. Important to these figures is the median weight of the largest resected parathyroid gland of 430 mg and a mean calcium value of 11.0 mg/dL (normal, 8.9 to 10.1 mg/dL). Intraoperative PTH monitoring was associated with a sensitivity, positive predictive value, and accuracy of 98 percent, 99 percent, and 97 percent, respectively. The true negative rate, defined as no decline in the PTH value when the patient still had an additional enlarged parathyroid gland, was 8 percent.”

Dr. McKenzie notes: “Of the patients undergoing focused parathyroidectomy, 70 percent received general anesthesia, and 30 percent had a combination of local anesthesia and intravenous sedation. Changes in anesthetic technique have allowed our patients undergoing general anesthesia to be discharged from the hospital early with much less nausea. Correspondingly, 89 percent of our patients were dismissed from the hospital as outpatients, whereas 11 percent were hospitalized, most often due to comorbidities necessitating in-hospital observation.”

Dr. Farley concludes: “The successful outcome of parathyroid surgery, specifically focused parathyroid exploration, depends on a centralized, highly skilled, experienced multidisciplinary team to achieve consistently successful outcomes.”

Figure 1. The small, 3-cm incision site is locally anesthetized.

Figure 2. Through this small incision, the lower poles of each thyroid lobe are seen.

Figure 3. The lower pole of the left thyroid lobe is elevated, and a small venous branch is clipped and cut. The parathyroid adenoma is seen, somewhat hidden in the portion of the thymus attached to the thyroid.

Figure 4. After the parathyroid adenoma has been removed, the incision is closed by suturing the muscles (A) and skin (B) with internal dissolvable sutures, and a small bandage is applied (C).
Melorheostosis

Melorheostosis (OMIM 155950) is a rare sporadic skeletal dysplasia of unknown cause that was first reported in 1922. This disorder is characterized by dense sclerotic bone in a sclerotomal distribution, following the pattern of osseous innervation. The term melorheostosis is derived from the Greek melos, meaning limb, rhein, meaning flowing, and ostosis, meaning bone formation. Melorheostosis causes thickening of cortical bone such that it looks, in classic cases, like melting wax dripping down a candle.

Bart L. Clarke, M.D., of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic’s campus in Rochester, Minnesota, says: “Melorheostosis may present at any age, but usually presents in childhood or adolescence, with bone lesions progressing most rapidly in childhood, but with occasional progression in adulthood. Both sexes appear to be affected equally. The onset of this disorder usually presents with limb deformity, contracture, bone or joint pain, joint stiffness or limited range of motion, or both, but pain and stiffness are the major symptoms. Pain is more prominent if subperiosteal bone formation continues. Soft tissue changes may be noted before the hyperostosis is recognized and occasionally in infancy.”

Peter J. Tebben, M.D., of the of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition and the Division of Pediatric Endocrinology and Metabolism in the Department of Pediatric and Adolescent Medicine at Mayo Clinic’s campus in Minnesota, adds: “The bones involved usually are in one limb only, but if bilateral, they are usually asymmetrical. Limb length discrepancy may develop due to asymmetric early fusion of epiphyses. Skin overlying affected bone may show thickening, shininess, fibrosis, erythema, linear scleroderma-like patches, hyperpigmentation, edema or hypertrichosis. Fibromas, fibrolipomas, capillary hemangiomas, lymphangiectasia and arterial aneuysms have been reported in some cases. Long bones are classically affected by melorheostosis. The legs are affected more often than the arms, with short bones of both the hands and feet also being affected. Rarely the skull or axial skeleton is affected. Melorheostosis may mimic other conditions such as myositis ossificans, osteoma or parosteal osteosarcoma. Other diseases that can be associated with melorheostosis include osteopoikilosis, osteopathia striata, scleroderma and Buschke-Ollendorff syndrome.”

The diagnosis of melorheostosis is clinical and radiographic. Dense, irregular and eccentric hyperostosis of both the periosteal and endosteal surfaces of a single bone, or several adjacent bones, is the classic presentation of melorheostosis (Figure 1). Bone may develop in soft tissues near skeletal lesions, especially near the joints, which can further limit mobility (Figure 2). Bone scans show increased uptake in involved bones due to increased blood flow to affected bones.

The increased uptake noted on bone scan also helps distinguish melorheostosis from osteopoikilosis and osteopathia striata, which do not show scintigraphic abnormalities. Laboratory studies in patients with melorheostosis show normal serum calcium, phosphorus and alkaline phosphatase. Serum bone alkaline phosphatase is usually normal unless a fracture or stress fracture has recently occurred.

Dr. Tebben notes: “The cause of melorheostosis remains unknown. It was initially thought that a mutation in the LEMD3 gene caused the disease, but further research showed that this mutation was associated with osteopoikilosis and
Buschke-Ollendorff syndrome instead. Research thus far has not yet found the gene causing this disorder. It has been postulated that an infection early in intrauterine development may predispose to melorheostosis. The distribution of the bone and soft tissue lesions within sclerotomes, myotomes and dermatomes suggests that the cause is due to mutation causing a segmentary embryonic change.”

Dr. Clarke explains: “Histopathology studies have shown increased osteoid formation, increased angiogenesis in affected bones, increased bone turnover and over production of bone matrix. During pubertal growth, endosteal thickening is characteristic, with periosteal new bone formation typical during adulthood. Affected bone is sclerotic, with thickened irregular lamellae. Marrow fibrosis may be seen. The collagen in the scleroderma-like skin lesions appears normal, distinctly different from classical scleroderma, and affected skin has therefore been classified as linear melorheostotic scleroderma. The treatment is largely surgical when contractures are present, but recurrence is common. There are no guidelines or clinical trials to guide medical treatment for painful hyperostosis, but anti-resorptive agents such as oral or intravenous bisphosphonates have been tried with variable success for pain control. Pain management is a significant issue for many patients.”

The Parathyroid-Bone Clinic staff at Mayo Clinic’s campus in Rochester, Minnesota, sees patients with melorheostosis and coordinates the multidisciplinary care with the Department of Orthopedic Surgery, Department of Physical Medicine and Rehabilitation, and the Pain Clinic. A multimodal approach is usually taken, with coordinated care emphasizing management of pain and functional limitations. Physicians from the Bone Clinic and other specialties at Mayo Clinic’s campus in Rochester, Minnesota, have been involved in organizing and hosting the Melorheostosis Association patient meeting in Rochester for a number of years.

Figure 2. Cortical thickening primarily on the periosteal surface of the distal femur and proximal fibula (closed arrow). Also noted are areas of dystrophic mineral in the soft tissues about the right knee and over the calf soft tissues consistent with melorheostosis (open arrows) in a 30-year-old woman.

Stereotactic Radiosurgery of Growth Hormone-Producing Pituitary Adenomas

Growth hormone (GH)-producing pituitary adenomas account for approximately 10 percent of all pituitary neoplasms and cause the clinical syndrome of acromegaly. Neena Natt, M.D., of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic’s campus in Rochester, Minnesota, says: “Untreated acromegaly is a morbid condition associated with disfigurement and increased mortality. Treatment options include surgical resection, medical therapy, radiotherapy, stereotactic radiosurgery or a combination of these approaches. The goals of treatment are to achieve normal blood levels of GH and insulin-like growth factor 1 (IGF-1), reverse the signs and symptoms related to GH excess, improve symptoms related to tumor mass effect, and preserve normal pituitary function.” Transsphenoidal surgery is generally considered the first line treatment for patients with acromegaly. Surgical resection can normalize hormone levels rapidly for the majority of patients (about 65 percent). Biochemical remission is achieved less frequently in patients with macroadenomas or in patients whose tumors extend into the cavernous sinus.

Bruce E. Pollock, M.D., of the Department of Neurologic Surgery at Mayo Clinic’s campus in Minnesota, explains: “Radiotherapy has long been regarded as a conventional adjuvant to surgical treatment or as primary treatment for inoperable tumors. Despite tumor growth control rates higher than 90 percent after radiotherapy of GH-producing tumors, the rate of biochemical remission is much lower and generally requires five or more years for the GH and IGF-1 levels to normalize. In addition, the majority of patients having radiotherapy to the sellar region develop some degree of hypopituitarism. Over the past 25 years, stereotactic radiosurgery (SRS) has emerged as an effective alternative or adjunct to
surgical resection and radiotherapy for patients with pituitary adenomas. Radiosurgery allows the delivery of focused radiation in a single session to the tumor with little radiation exposure to the surrounding normal structures.”

**Patient selection**

Proper patient selection is the most important factor associated with good outcomes after radiosurgery. Generally, patients with pituitary adenomas with considerable suprasellar extension are typically not considered good candidates for radiosurgery because patients with larger lesions often have visual loss related to mass effect. Although radiosurgery does result in growth control and size reduction in the majority of pituitary adenomas, these effects occur gradually over several years. Therefore, surgical resection is the preferred approach for patients with large pituitary adenomas. Dr. Pollock says: “For many patients, complete tumor removal is not possible because the tumor extends into the cavernous sinus. In these patients, radiosurgery can be part of a staged approach with transsphenoidal surgery. Initially, the tumor is debulked to create a separation between the top surface of the tumor and the optic apparatus, without an attempt at resection of the tumor involving the cranial nerves, major arteries or dural venous sinuses. Radiosurgery can then be performed for the remaining tumor volume with little risk of cranial nerve deficits. This multimodality treatment approach reduces patient morbidity and improves long-term tumor control.”

Dr. Natt notes: “Our center and others have determined that the results of pituitary adenoma radiosurgery are adversely affected by the use of pituitary suppressive medications (bromocriptine, cabergoline, octreotide) at the time of radiosurgery. Consequently, we have patients discontinue these medications at least eight weeks before radiosurgery.”

**Radiosurgery with the Gamma Knife**

Radiosurgery is performed at Mayo Clinic using the Leksell Gamma Knife Perfexion (Elekta Inc., Atlanta, Georgia). The Gamma Knife has been used for more than four decades to treat more than 800,000 patients worldwide. Radiosurgery is an outpatient procedure, performed with the patient under local anesthesia, and requires virtually no recovery time. After placement of a stereotactic head frame, the patient has an MRI and a CT performed for dose-planning purposes. A dose plan is then created and reviewed by a neurologic surgeon, a radiation oncologist and a radiation physicist (Figure).

Between January 1990 and December 2013, 443 patients with pituitary adenomas had undergone SRS at Mayo Clinic — from a total clinical experience that exceeds 6,000 patients. Of the 443 patients who underwent SRS, 150 patients (34 percent) have had acromegaly. Ninety percent of the patients have undergone prior surgery; 80 percent had tumors with extension into the cavernous sinus. Dr. Pollock notes: “To minimize the incidence of visual deficits after radiosurgery, we limit the radiation dose to the optic nerves to less than 12 Gy. This dose prescription has resulted in visual morbidity of less than 1 percent in patients who have not received prior radiation. To date, no patient with a GH-producing pituitary adenoma has had tumor growth after radiosurgery with follow-up that now extends beyond 20 years.”

Two variables correlate with biochemical remission after radiosurgery in patients with acromegaly (Table). Dr. Pollock explains: “First, patients with IGF-1 levels less than 2.25 times the upper limit of normal (ULN) are almost three times more likely to achieve biochemical remission than are patients with IGF-1 levels that are more than or equal to 2.25 times the upper limit of normal (ULN).”

Figure. Postgadolinium fused MRI-CT of a patient with persistent acromegaly after prior transsphenoidal surgery showing tumor extending into the right cavernous sinus. The tumor receives a minimum dose of 25 Gy (yellow line) and a maximum dose of 50 Gy. The maximum dose to the adjacent right optic nerve is 79 Gy. The green line represents the volume of tissue receiving 10 Gy of radiation.
Results of Radiosurgery for Patients With Acromegaly

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<th>Biochemical remission</th>
<th>Outcome</th>
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<td>Overall</td>
<td>60 percent at 5 years</td>
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<tr>
<td>IGF-1 &lt; 2.25 times ULN</td>
<td>79 percent at 5 years</td>
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<tr>
<td>IGF-1 ≥ 2.25 times ULN</td>
<td>29 percent at 5 years</td>
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<tr>
<td>Off suppressive medications</td>
<td>91 percent at 5 years</td>
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<tr>
<td>On suppressive medications</td>
<td>38 percent at 5 years</td>
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<tr>
<td>New anterior pituitary deficits</td>
<td>33 percent at 5 years</td>
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Table. Biochemical remission was defined as a fasting serum GH concentration of less than 2 ng/mL and normal age- and sex-adjusted serum IGF-1 concentration off all suppressive medications. IGF-1, insulin-like growth factor 1; ULN, upper limit of normal.

of normal. Second, patients who were not taking pituitary suppressive medications for at least one month before radiosurgery were more than four times more likely to have biochemical remission. The median time to biochemical remission was three years, although endocrine normalization has been noted beyond five years in some cases. New anterior pituitary deficits occurred in 33 percent of patients five or more years after radiosurgery. The most frequent anterior pituitary deficit was hypogonadism. Biochemical remission is possible in 80 to 90 percent of properly selected patients with acromegaly.”

Education Opportunities

15th Annual Mayo Clinic Nutrition and Wellness in Health and Disease
Sept. 25-26, 2015, Washington, D.C.
Nutrition, physical activity and other healthy lifestyle behaviors are vital components in the promotion of health and the treatment of disease. This course — designed for physicians, advanced practice clinicians, dietitians, nurses, and health and wellness staff — will provide a full-spectrum, in-depth overview of situations and topics that clinicians encounter in the ambulatory setting, including obesity in adults and children, weight management strategies, obesity-associated medical conditions, the role of healthy diets, bariatric surgery and pre- and post-surgery medical management, prevention of common medical conditions through healthy lifestyles, effective ways to provide coaching, nutrition for selected groups, nutrition topics in the news, behavior modification, and resilience, in addition to physical activity and wellness. A culinary demonstration will highlight cooking techniques to prepare healthy, great-tasting food. Current clinical topics will be highlighted through presentations that offer practical clinical management pearls, interactive case studies and panel discussions. The course will be held at The Mayflower Renaissance Washington, D.C., Hotel. For more information about this course, please visit www.Mayo.edu/cme/endocrinology or call 800-323-2688 (toll-free). Course hashtag: #MayoNutrCME