Tumors, IGF-2, and Hypoglycemia: Insights From the Clinic, the Laboratory, and the Historical Archive

Yevgeniya Dynkevich, Kristina I. Rother, Ian Whitford, Sana Qureshi, Sneha Galiveeti, Alessandra L. Szulc, Ann Danoff, Tracy L. Breen, Nargess Kaviani, Michael H. Shanik, Derek LeRoith, Riccardo Vigneri, Christian A. Koch, and Jesse Roth

Queens Long Island Medical Group (Y.D.), North Shore-Long Island Jewish (NS-LIJ) Health System, Hicksville, New York 11801; National Institute of Diabetes and Digestive and Kidney Diseases (K.I.R.), Bethesda, Maryland 20892; Laboratory of Diabetes and Diabetes-Related Research (I.W., S.Q., S.G., J.R.), Feinstein Institute for Medical Research, NS-LIJ Health System, Manhasset, New York 11030; Albert Einstein College of Medicine (A.L.S., J.R.), Yeshiva University, Bronx, New York 10461; Division of Endocrinology, Diabetes, and Metabolism (A.D.), New York University School of Medicine, New York, New York 10016; Veterans Administration New York Harbor Healthcare System (A.D.), New York, New York 10010; Division of Endocrinology (T.L.B.), NS-LIJ Health System, Great Neck, New York 11021; Hofstra NS-LIJ School of Medicine (T.L.B., J.R.), NS-LIJ Health System, Manhasset, New York 11549; Endocrinology and Metabolism (N.K.), Geisinger Health System, State College, Pennsylvania 16801; Endocrine Associates of Long Island (M.H.S.), P.C., Smithtown, New York 11787; Stony Brook University Hospital (M.H.S.), Stony Brook, New York 11794; Division of Endocrinology, Diabetes, and Bone Diseases (D.L.), The Samuel Bronfman Department of Medicine, Mt. Sinai School of Medicine, New York, New York 10029; Diabetes and Metabolism Clinical Research Center of Excellence (D.L.), Clinical Research Institute at Rambam, Haifa, Israel 31096; Endocrine Unit (R.V.), Department of Clinical and Molecular Biomedicine, University of Catania, Garibaldi-Nesima Hospital, Catania, and Istituto di Biostrutture e Bioimmagini-Consiglio Nazionale delle Richerche Catania Section, Catania, Italy 95131; and Division of Endocrinology (C.A.K.), Department of Medicine, University of Mississippi School of Medicine, Jackson, Mississippi 39216

Tumors of mesenchymal and epithelial origin produce IGF-2, which activates pathways in the tumors. In a minority of patients, the tumors (hepatomas, fibromas, and fibrosarcomas are the most common among many) release into the circulation enough IGF-2–related peptides to mimic the fasting hypoglycemia characteristic of patients with insulin-producing islet-cell tumors. Rarely, markedly elevated IGF-2 levels produce somatic changes suggestive of acromegaly. Typically, the elevated IGF-2 levels are associated with suppressed plasma levels of insulin, IGF-1, and GH. Complicating the pathophysiology are the IGF binding proteins (IGFBPs) that can bind IGF-2 and IGF-1, modifying hormone metabolism and action. IGFBP concentrations are often altered in the presence of these tumors. At the cellular level, the 3 hormone-related ligands, IGF-2, IGF-1, and insulin, all bind to 4 (or more) types of IGF-1 receptor (IGF-1R) and insulin receptor (IR). Each receptor has its own characteristic affinity for each ligand, a tyrosine kinase, and overlapping profiles of action in the target cells. The IGF-2R, in addition to binding mannose-6-phosphate–containing proteins, provides an IGF-2 degradation pathway. Recent evidence suggests IGF-2R involvement also in signal transduction. Surgery, the treatment of choice, can produce a cure. For patients not cured by surgery, multiple therapies exist, for the tumor and for hypoglycemia. Potential future therapeutic approaches are sketched. From 1910 to 1930, hypoglycemia, insulin, insulinomas, and non–islet-cell tumors were recognized. The latter third of the century witnessed the emergence of the immunoassay for insulin; the IGFs, their binding proteins, and assays to measure them; and receptors for the insulin-related peptides as well as the intracellular pathways beyond the receptor. In closing, we replace non–islet-cell tumor hypoglycemia, an outdated and misleading label, with IGF-2-oma, self-explanatory and consistent with names of other hormone-secreting tumors. (Endocrine Reviews 34: 798–826, 2013)
C. IGF-2 measurements  
V. IGF-2 Physiology  
VI. Control of IGF-2 Production  
VII. IGF-2 Binding to IGF-1 Receptors and IRs  
VIII. IGF-2 Receptor  
IX. IGF Binding Proteins  
X. Mechanisms Leading to Hypoglycemia With IGF-2-oma  
XI. Other Big IGF-2–Linked Syndromes  
XII. Tumor-Directed Therapies  
XIII. Hypoglycemia-Directed Therapies  
XIV. Future Therapies  
A. Tumor-directed therapies (future)  
B. Hypoglycemia-directed therapies (future)  
XV. Relevant History  
A. What's in a name?  
B. Hypoglycemia  
C. Major IGF-2-oma landmarks  
D. Pioneer studies  
E. Emergence of hypoglycemia  
F. Emergence of the signs of hypoglycemia  
G. Plasma insulin measurements before 1960  
H. Radioimmunoassay of insulin  
I. Extant theories before 1974  
J. Megyesi, NIH, and the 1974 Watershed

I. Introduction

Non–islet-cell tumor hypoglycemia (NICTH), denotes the syndrome of hypoglycemia produced by or associated with any neoplasm other than an insulinoma. The underlying mechanism of hypoglycemia in nearly all patients with this syndrome is overproduction of IGF-2 by the tumor, which includes mature IGF-2 and incompletely processed forms of IGF-2, referred to collectively as big IGF-2. The IGF-2–secreting tumors are derived from an extraordinarily broad range of tissue types. We introduce the term IGF-2-oma to describe these tumors.

The clinical consequences of IGF-2 excess include 1) autocrine-paracrine stimulation of tumor cells by IGF-2 (Figure 1); 2) spontaneous hypoglycemia, typically with fasting, that closely resembles the hypoglycemia characteristic of functioning islet-cell tumors but without elevated insulin levels; and 3) in rare patients, (acromegoidal) features (in the absence of elevated GH or IGF-1). This review of IGF-2–linked tumor hypoglycemia, with emphasis on the diagnosis, pathophysiology, and treatment, is a completely revised version of an earlier review (1).

II. Epidemiology

The initial recognition of this syndrome dates to 1929 in patients with hepatocellular carcinoma (2, 3). Since then, many other types of neoplasms have been associated with the IGF-2-oma syndrome. Although the true incidence is unknown, it is generally believed to be much less frequent than hypoglycemia from insulinomas. Epidemiologic data challenges this assumption and suggests that IGF-2-oma–induced hypoglycemia may be substantially more common. In the developing world, hepatitis B and hepatitis C infections are very widespread; both significantly increase the risk for hepatocellular carcinoma, a leading form of cancer worldwide. Hypoglycemia has been increasingly recognized in patients with hepatocellular carcinoma (Table 1) (4).

Most commonly, IGF-2–linked hypoglycemia has been observed in patients with solid tumors that are either of mesenchymal or epithelial origin. A handful of new case reports appear each year (Table 1) (5–65, 146). Among tumors of epithelial origin, hepatocellular carcinomas dominate the etiology, with adrenocortical carcinomas a distant second. Mesenchymal tumors include neoplasms that arise from fibroblasts and fibrous tissue (eg, fibrosarcoma), from endothelium (eg, hemangiopericytoma), blood cells (eg, leukemia, lymphoma), and myogenic cells (eg, rhabdomyosarcoma and leiomyosarcoma). The most common mesenchymal tumors associated with hypoglycemia are fibrosarcomas and mesotheliomas (Table 1). Frequently, these tumors are quite large at the time of diagnosis, weighing 3 kg or more. The mass effect of the tumor depends on size and location. One of the largest reported fibrous tumors weighed more than 20 kg (59).

Tumors that arise in the thorax, retroperitoneum, or pelvis are usually clinically silent and tend to reach a significant size before diagnosis. On the other hand, lesions that are located in the extremities, eg, in the thigh or knee, are detected much earlier due to signs and symptoms related to mass. Overall, mesenchymal tumors are generally slow-growing. Hypoglycemia by itself is not a predictor of the size or aggressiveness of the tumor and occurs with benign and malignant tumors (60, 61).

III. Clinical Presentation

Nearly all insulinoma patients and about half of patients with IGF-2–linked tumor hypoglycemia present with clinical findings related to hypoglycemia (66). In about half of patients with IGF-2–linked hypoglycemia, the neoplasm is diagnosed (often long) before the onset of hypoglycemia (40, 67). In these patients, the most common presenting features are weight loss, abdominal mass, pain, or detection on routine radiologic examination (Figure 2) (40, 59).
A. Hypoglycemia

As in patients with insulinoma, hypoglycemia associated with an IGF-2–producing tumor typically presents in the fasting state (40). Although both neuroglycopenic and autonomic symptoms of hypoglycemia may occur, cerebral dysfunction dominates, as is typical with frequently repeated bouts of hypoglycemia or hypoglycemia that comes on slowly over a period of hours. An early sign of neuroglycopenia is the loss of good judgment, so that the patient ceases to be a reliable steward of his/her own well-being. The patient may present with confusion, amnesia, or frank psychosis (40, 59, 68). Hypoglycemia, when severe or protracted, can cause seizures, coma, and death (69). Therefore, along with cerebral metastases and drugs, hypoglycemia due to IGF-2 should be considered in any cancer patient with new alterations in behavior or mental status (50). Hypoglycemia may produce focal neurological signs and symptoms, mimicking a stroke or metastases (70). It is likely that IGF-2–linked tumor hypoglycemia remains unrecognized in many cancer patients who are receiving palliative care, where neuroglycopenic symptoms could be erroneously attributed to effects of narcotics or other medications. Symptoms related to catecholamine release, including sweating, anxiety, and tremor, may occur but can be blunted or absent in the setting of repeated episodes of hypoglycemia or hypoglycemia of slow onset.

For a given level of glucose, patients with IGF-2–producing tumors (or insulinomas) typically are more impaired than other patients with identical blood glucose levels, eg, hypoglycemia that is associated with a prolonged fast in a normal person that quickly results in a rapid fall in insulin to very low levels (71–75). The brain depends largely on glucose and/or ketones for its energy. With fasting, the fall in glucose levels is offset by a rise in β-hydroxybutyrate and other ketones (75, 76). With hypoglycemia brought about by activation of insulin-related receptors (eg, with IGF-2 or insulin), the rise in ketones is blunted (76), magnifying the brain’s energy shortage. The continuing activation of insulin receptors (IRs) by IGF-2 (or insulin) leads to 1) continued glucose utilization especially by skeletal muscle as well as 2) suppression of free fatty acid release by adipocytes and inhibition of glucose release, glycolysis, gluconeogenesis, and ketogenesis in the liver (77–79). In addition, both glucagon and GH release are suppressed by IGF-2 (Figure 3) (80, 81). The suppression of energy-producing substrates and of counterregulatory hormones heightens the vulnerability to hypoglycemia.

Elevated levels of IGF-2 also suppress normal insulin secretion by pancreatic β-cells (141, 281, 285), which may lead to postprandial hyperglycemia in the setting of fasting hypoglycemia, similar to patients with insulinoma (62). Low potassium levels have been observed in about half of patients with IGF-2–linked hypoglycemia (40).

B. Acromegaloid changes

In addition to effects on glucose metabolism, IGF-2omas in rare instances can generate visible growth-pro-
motivating changes. Trivedi et al (63) reported a woman with a pelvic clear cell sarcoma and acromegalic features including skin tags and coarse facial appearance who postoperatively experienced disappearance of the acromegalic features. Skin lesions such as seborrheic keratoses, skin tags, and rhinophyma have all been described in patients with IGF-2–producing tumors (32, 64, 65). Although hypoglycemia is ascribed largely to IGF-2 activation of the IR, the acromegalic features are thought to arise largely from IGF-2–mediated stimulation of multiple subclasses of insulin-related and IGF-1–related receptors. Note that this unusual combination of acromegalic features and tumor-associated hypoglycemia may not be unique to IGF-2-oma. The same combination may possibly occur in rare patients with multiple endocrine neoplasia (MEN) type 1 who have both insulin-secreting and GH-secreting tumors (82).

### Table 1. Tumors Associated With IGF-2–Induced Hypoglycemia

<table>
<thead>
<tr>
<th>Tumor Type</th>
<th>Prevalence, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Epithelial origin</td>
<td>45</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>20</td>
</tr>
<tr>
<td>Adrenocortical carcinoma</td>
<td>5</td>
</tr>
<tr>
<td>Stomach</td>
<td>4</td>
</tr>
<tr>
<td>Pancreas (non-islet cell)</td>
<td>4</td>
</tr>
<tr>
<td>Lung</td>
<td>3</td>
</tr>
<tr>
<td>Colon, rectum, esophagus</td>
<td>3</td>
</tr>
<tr>
<td>Carcinoid, neuroendocrine, medullary thyroid</td>
<td>2</td>
</tr>
<tr>
<td>Breast, ovary, prostate</td>
<td>1</td>
</tr>
<tr>
<td>Others: seminoma, pseudomyxoma, sarcomatous teratoma, melanoma, Wilms’ tumor, dysergeminoma of the ovary, cervix, bladder, uterus, cholangioma</td>
<td>3</td>
</tr>
<tr>
<td>Mesenchymal origin</td>
<td>42</td>
</tr>
<tr>
<td>Fibrosarcoma, fibroma</td>
<td>23</td>
</tr>
<tr>
<td>Mesothelioma</td>
<td>8</td>
</tr>
<tr>
<td>Hemangiopericytoma, hemangiendothelioma, hemangiosarcoma</td>
<td>7</td>
</tr>
<tr>
<td>Hematologic: lymphoma, leukemia, lymphosarcoma, myeloma</td>
<td>1</td>
</tr>
<tr>
<td>Others: rhabdomyosarcoma, liposarcoma, neurofibroma, neurofibrosarcoma, histiocytoma, neuroblastoma, mesoblastic nephroma, neurilemroma, meningioma, reticulum cell sarcoma, pelvic clear cell sarcoma</td>
<td>3</td>
</tr>
<tr>
<td>Unknown etiology</td>
<td>13</td>
</tr>
</tbody>
</table>

* One systematic study of patients with hepatocellular carcinoma (before the introduction of IGF-2 and its assays) distinguished 2 types of tumors. In 124 of their 142 patients, the tumor was poorly differentiated and rapidly growing associated with profound weakness and muscle wasting. Of these, 21 (within 2 weeks of death) developed fasting hypoglycemia with slow decreases in glucose concentrations that were easily controlled. It is speculated that the hypoglycemia was predominantly due to destruction of liver. In the other 18 patients, the tumor was slow growing, well differentiated, and without wasting of skeletal muscle. Within 2 to 10 months before death, they manifested precipitous falls in blood glucose that were difficult to control. The tumors in these patients were likely to be releasing IGF-2–related molecules, producing the hypoglycemia.

Adapted from Refs. 1, 77, 213, 228, 286, and 287.

### IV. Diagnosis

Hypoglycemia in a patient with diabetes is quite common and typically related to effects of glucose-controlling medications especially with intensive insulin regimens. The timely recognition of IGF-2-omas (or insulinomas) arising in patients with preexisting diabetes can be very challenging. Surreptitious (or factitious) administration of insulin (or other glucose-lowering medications) should always be considered in the differential diagnosis of fasting hypoglycemia, especially in patients with diabetes (or with a household relative with diabetes). Patients who surreptitiously self-administer insulin (or other medications) often have a psychiatric disorder, intend self-harm, and may be suicide risks (83–85).

#### A. Fasting hypoglycemia

In a nondiabetic patient, fasting hypoglycemia is rare and warrants thorough evaluation. An algorithm for the management of a patient with hypoglycemia has been published by The Endocrine Society (74). The evaluation should be initiated with the documentation of true hypoglycemia. Inexperienced professionals may erroneously begin the workup of hypoglycemia based on symptoms or signs suggesting hypoglycemia without first demonstrating low blood glucose. The next step is to document all components of Whipple’s triad (86, 87): 1) symptoms and/or signs of hypoglycemia, 2) low plasma glucose, and 3) resolution of signs and symptoms upon reversal of hypoglycemia. This allows physicians to exclude artificial causes of low blood glucose before embarking on further workup. For instance, low measured blood glucose in the absence of any related symptoms may be a result of improper sample collection (wrong tube) or incorrect processing in the laboratory. Artificially low blood glucose measurements (pseudohypoglycemia) have been reported in patients with blood dyscrasias (eg, leukemia or polycythemia vera); in the test tube, the elevated number of blood cells consumes excessive amounts of glucose (88, 89).

A further rational approach to the evaluation of hypoglycemia is suggested in Tables 2 and 3. After confirmation of hypoglycemia, the next step is to evaluate carefully the patient’s history and physical findings to identify any systemic conditions that may be causing or contributing to hypoglycemia. These include first and foremost cortisol deficiency; hypoglycemia can be the presenting finding in Addison’s disease. Other conditions include hypopituitarism as well as critical illnesses with liver failure and/or kidney failure, sepsis, GH deficiency, and severe starvation. Ethanol is known to inhibit gluconeogenesis in the liver, contributing to hypoglycemia. Again, the possibility...
of drug-induced hypoglycemia must be seriously considered in the differential diagnosis (Table 2) (83).

When no identifiable cause of hypoglycemia has been found, the next step is to measure plasma insulin and C-peptide, along with the corresponding glucose level during an episode of hypoglycemia, either spontaneous or provoked by a supervised fast (Table 3). In some cases, plasma levels of proinsulin and β-hydroxybutyrate will prove to be helpful. For the patient who experiences fasting hypoglycemia, a supervised fast lasting up to 72 hours, if necessary, should be performed. Although the fast is traditionally carried out in an inpatient setting, with careful planning and excellent supervision, the fast can be performed in a well-supervised outpatient facility. Most patients with insulinoma manifest hypoglycemia within the first 24 to 36 hours of fasting (90). Similar data with IGF-2-omas have not been compiled. Continually asking patients to perform simple calculations, eg, serial sevens, is a convenient bedside monitor of the progression of the hypoglycemia.

Hyperinsulinemic hypoglycemia (with corresponding C-peptide measurement) in the absence of antibodies to insulin and negative drug screens for sulfonylureas and other insulin secretagogues suggest the presence of an insulinoma. However, a similar pattern may be seen with 1) a rare condition, noninsulinoma pancreatogenous hypoglycemia syndrome (91), and 2) as an uncommon side effect of the increasingly popular gastric bypass surgical procedures, but not with all bariatric surgical procedures (92–94). Post-gastric bypass surgery nesidioblastosis may have underlying genetic defects that can explain why only a small subset of such surgically treated patients develop hypoglycemia (292). Most often hypoglycemia occurs postprandially. The suggested treatment is medical therapy with diazoxide and/or octreotide, or partial pancreatectomy for patients refractory to medical therapy (91–94, 293).
B. Autoimmune hypoglycemia

Hypoglycemia due to autoimmunity is rare, caused by either of two types of antibodies, one to insulin, and the other to the IR. Both types of antibodies can be measured. Patients with IR antibodies typically present with extreme insulin resistance (often with hyperglycemia), due to the initial blocking action of these antibodies. They frequently also have manifestations of another autoimmune disorder such as lupus erythematosus as well as acanthosis nigricans, a skin lesion often linked to very high levels of circulating insulin. The receptor antibodies can also act as agonists to the IR, causing hypoglycemia. Patients with this condition have high insulin levels and disproportionately low C-peptide levels (95). On the other hand, in patients with antibodies to insulin, the circulating levels of insulin are usually increased together with C-peptide. These antibodies bind insulin; over a prolonged period of time, the insulin is slowly released, causing hypoglycemia. Antibodies to insulin have been described in patients with myeloma (96, 97) and antibodies to the IR in Hodgkin’s lymphoma (98).

C. IGF-2 measurements

IGF-2–linked tumor hypoglycemia is suspected when hypoglycemia without hyperinsulinemia is present and the other etiologies mentioned above have been ruled out. Typically, insulin, proinsulin, C-peptide, and β-hydroxybutyrate levels are low (40). Unlike brief bouts of hypoglycemia that often produce an acute elevation in GH, in these patients, GH concentrations are usually low along with low plasma levels of total IGF-1 (40). Levels of total IGF-2 may be elevated or normal, whereas levels of the IGF-2 precursors are often elevated (99). An elevated ratio of IGF-2 to IGF-1 may be helpful in the diagnosis. The normal molar ratio of IGF-2 to IGF-1 in plasma is about 3:1. In a patient with recurrent hypoglycemia, ratios of >10:1 are virtually diagnostic for IGF-2–linked hypoglycemia. Fukuda et al (100) found IGF-2 to IGF-1 ratios as high as 64:1. The IGF-2 to IGF-1 ratio is particularly helpful when IGF-2 levels are within the normal range (101). Abnormally high IGF-2 to IGF-1 ratios can also occur in patients with sepsis and severe cachexia (102, 103). However, in the latter 2 conditions, both IGF-2 and IGF-1 levels are subnormal (104). With IGF-2–linked hypoglycemia, IGF binding protein (IGFBP)-1 and IGFBP-2 levels are increased. Low GH levels lead to reduction in synthesis of IGFBP-3 and of the acid-labile subunit (ALS) (Figure 3). The low levels of ALS lead to a shift in the molecular distribution of protein-bound IGFs from predominantly...
150-kDa complexes toward 50-kDa complexes (see Section IX and Figure 4). Several radioimmunoassays have been developed to measure pro–IGF-2, using antibodies against the E-domain (105–108). Daughaday and Trivedi (105) described a radioimmunoassay that selectively detected the first 21 residues of the E-domain (E1–21) of pro–IGF-2 (68–88). Similarly, Liu et al (107) developed an assay against pro–IGF-2 (69–84) and Tally et al (108) reported a radioimmunoassay for a 15–amino-acid segment of the E-domain. Most recently, a more rapid assay has been developed that employs thin layer chromatography (109). The assays for pro–IGF-2 are not readily available commercially but are offered at several research institutions.

Other causes of IGF-2-oma hypoglycemia are possible but very rare. Over the years there have been reports of tumors of nonpancreatic origin secreting insulin, but in most, the data are not robust. The most convincing is that of a very well-studied patient with a small-cell carcinoma of the cervix where the insulin, C-peptide, and proinsulin levels were elevated while IGF-1 and IGF-2 were not (110). The tumor itself had evidence for the production of insulin and other hormones typical of the pancreas.

A well-documented patient with recurrent hypoglycemia associated with elevated blood levels of IGF-1 (an IGF-1-oma) but not of IGF-2 or of insulin has been reported. IGF-1 levels on the binding proteins were also elevated. Combined GH and glucocorticoid treatment relieved the hypoglycemia, whereas the IGF-1 levels were unchanged. Chemotherapy caused tumor regression, normalization of IGF-1, and disappearance of the hypoglycemia (54).

V. IGF-2 Physiology

In humans during fetal life, IGF-2 plays an important role in cell proliferation and apoptosis. Its expression increases postnatally (111, 112). In human adults, IGF-2 is expressed in the liver, driven by a liver-specific promoter that is absent in rodents (Table 4) (113–116). Initially, the role of IGF-2 in human adults was missed because its role in rodents was limited to fetal development. IGF-2-omas have also been demonstrated in dogs (117).

VI. Control of IGF-2 Production

The Igf2 gene (structurally homologous to the Insulin gene) lies on the short arm of chromosome 11 (11p15.5) between the genes for insulin and H19 (118). The Igf2 gene is expressed only from the paternally inherited allele. The H19 gene, coexpressed with Igf2 in the same embryonic tissues, is only maternally expressed (113, 119). The genetic imprinting of Igf2 begins in the 8-cell preimplantation embryo and occurs in all adult tissues except the choroid plexus and leptomeninges where Igf2 is biallelically expressed (120, 121). Control of the imprinting is governed by the imprinting control region, upstream of H19, composed of differentially methylated regions that have insulators, silencers, and activators at their partitions. When this region is methylated, as it is on the paternal chromosome, CCCTC-binding factor is unable to bind and so gene expression occurs. When this imprinting controlling region is unmethylated, as occurs on the maternally derived chromosome, it binds the vertebrate enhancer blocking protein, CCCTC-binding factor, thereby blocking the activity of the enhancers and Igf2 gene expression. When the maternal imprinting-controlling region is methylated, there is a loss imprinting of (H19 and) Igf2, as has been observed in many tumors (65, 122–131).

The Igf2 gene is translated into the pre-pro–IGF-2 peptide in a complex manner. The gene contains 9 exons; transcription is controlled by 5 promoters that lead to

---

**Table 2. Differential Diagnosis of Hypoglycemia**

<table>
<thead>
<tr>
<th>Work-up of a Patient With Low Blood Glucose</th>
</tr>
</thead>
<tbody>
<tr>
<td>A. Confirm Whipple’s triad before embarking on extensive work-up</td>
</tr>
<tr>
<td>1. Glucose ≤50 mg/dL (2.77 mmol/L)</td>
</tr>
<tr>
<td>2. Clinical manifestations of hypoglycemia</td>
</tr>
<tr>
<td>3. Resolution of symptoms upon treatment of hypoglycemia</td>
</tr>
<tr>
<td>B. Exclude identifiable systemic causes before proceeding</td>
</tr>
<tr>
<td>1. Hormone deficiencies</td>
</tr>
<tr>
<td>a. Primary adrenal insufficiency</td>
</tr>
<tr>
<td>b. Hypopituitarism with secondary adrenal insufficiency</td>
</tr>
<tr>
<td>2. Critical illness</td>
</tr>
<tr>
<td>a. Severe renal, hepatic or cardiac failure</td>
</tr>
<tr>
<td>b. Sepsis</td>
</tr>
<tr>
<td>3. Starvation</td>
</tr>
<tr>
<td>4. Drugs: salicylates, quinine, pentamidine, alcohol, accidental or surreptitious intake of insulin, and oral hypoglycemia agents</td>
</tr>
<tr>
<td>C. Focus on insulin-related hypoglycemia (insulinoma, post-gastric bypass hypoglycemia, noninsulinoma pancreatogenous hypoglycemia, anti-insulin antibodies, and antibodies against insulin receptor)</td>
</tr>
<tr>
<td>1. Measure glucose, insulin, pro-insulin, C-peptide, β-hydroxybutyrate, and glucose at the time of suspected hypoglycemia</td>
</tr>
<tr>
<td>2. Measure blood levels of antibodies against insulin and against the insulin receptor</td>
</tr>
<tr>
<td>3. Obtain hypoglycemic agent screen panel (see Table 3 for interpretation of findings)</td>
</tr>
<tr>
<td>D. Focus on Igf2-omas in patients with hypoglycemia with low insulin, C-peptide, pro-insulin, and β-hydroxybutyrate levels</td>
</tr>
<tr>
<td>1. Measure levels of pro–IGF-2: elevated level expected</td>
</tr>
<tr>
<td>2. Look for depressed levels of IGF-1, GH, and IGFBP-3, any of which would support the diagnosis</td>
</tr>
<tr>
<td>3. Measure total IGF-2: elevated level helpful; normal level is not exclusionary</td>
</tr>
<tr>
<td>4. Search for large tumors, especially in the thorax and retroperitoneum: large tumors supportive of diagnosis; small tumors may or may not be related to hypoglycemia</td>
</tr>
</tbody>
</table>

---

*a* Adapted from Ref. 288.

*b* Caution: anti-insulin antibodies can interfere with many methods of measurement of plasma insulin. Consult with experts to help you decide how antibodies to insulin in patient’s blood can be circumvented in the measurement of endogenous circulating insulin.
different effects on gene expression in specific tissues at various stages of development (118, 132). The promoters (P1–P4) are located in front of exons 1, 4, 5, and 6 (118). P0, which was discovered last, is located in front of exon 2 and is involved in human development in utero and in tumorigenesis (132). P1 is expressed in the human adult liver and is not expressed in rodents (Table 4) (114, 133, 134). P2, P3, and P4 are fetal promoters; P3 and P4 are repressed at birth, although they may be active in tumors. The different promoters produce distinct mRNA transcripts ranging in size from 2.2 to 6.0 kb. In addition, a smaller 1.8 kb transcript is derived from endonucleolytic cleavage at the 5′-untranslated region (UTR) of exon 9 and a 5′ cleavage product is formed from cleavage at the 5′-UTR (135).

Translation of the mRNA transcripts is regulated by leader sequences. Each promoter-specific mRNA transcript contains a different leader sequence on exons 1–3, 4, 5, and 6 (L1–L4) in the 5′-UTR. These leader sequences regulate mRNA translation and cleavage. L1, L2, and L4 allow efficient translation, whereas L3 represses translation (136, 137). L1 and L3 lead to more efficient cleavage of mRNA than L2 and L4. Therefore, the effect of the leader sequences on translation and cleavage appear to be independent (135). The significance of the 3′ and 5′ cleavage products is unclear, but increased levels of the 3′ cleavage product are associated with decreased levels of IGF-2 mRNA, suggesting that it may downregulate IGF-2 expression. It is also possible that the 3′ cleavage product may influence cell proliferation under certain conditions (135, 138). The 5′ cleavage product contains the IGF-2 mRNA coding region but lacks the polyadenine tail; as a result, it is very unstable and not translated (135).

The translation product of the Igf2 gene is a 180–amino-acid pre-pro–IGF-2 (Figure 5), consisting of an N-terminal 24–amino-acid peptide, the 67–amino-acid mature IGF-2, and an 89–amino-acid C-terminal extension, designated the E-domain. Posttranslational modifications of pre-pro–IGF-2 occur in the Golgi (139). The N-terminal sequence is removed and sialic acid-containing oligosaccharides are added by O-linkage to 1 or more threonine residues of the E-domain. The E-domain then undergoes proteolysis (140). During this process, the relatively stable intermediate pro–IGF-2E is formed (the mature IGF-2 with part of the E-domain attached). Normally, a small fraction of this intermediate may be secreted. In IGF-2-omas, abnormal processing often occurs, resulting in increased production and secretion of pro–IGF-2E (or big IGF-2) (35, 141).

Mature IGF-2 is a single-chain polypeptide, similar to IGF-1 and proinsulin. All 3 contain a B and A domain. The connecting piece of proinsulin is excised, but IGF-2 and IGF-1 retain the connecting piece (C-domain) as well as a short (C-terminal) D-domain not found in proinsulin (1, 5). Like IGF-1, the circulating level of IGF-2 is in the nanomolar range (normal range, 521–873 ng/ml), whereas that of insulin is in the picomolar to nanomolar range (normal range, 40–145 pmol/L). Unlike IGF-1 and IGFBP-3 production, which are responsive to GH, IGF-2 secretion is largely independent of GH action (115). IGF-2 interacts with GH and IGF-1 secretion through negative feedback mechanisms (99); in patients with IGF-2-omas, elevated plasma levels of IGF-2 are associated with depressed levels of GH and IGF-1 (and IGFBP-3) (1).

### VII. IGF-2 Binding to IGF-1 Receptors and IRs

IGF-2 (as well as IGF-1, insulin, and other insulin-related peptides) binds to a series of insulin-related receptors (Figure 6). IGF-2 signaling occurs mostly via the IGF-1 receptor (IGF-1R) (Figure 7), but also to a significant extent through interaction with the IR isofom A (IR-A). All of the IGF-1 and insulin-related receptors have a pair of ex-

---

**Table 3. Patterns of Findings During Fasting or After a Mixed Meal in Individuals With Hypoglycemia**

<table>
<thead>
<tr>
<th>Symptoms, Signs, or Both</th>
<th>Glucose (55 mg/dL)</th>
<th>Insulin (3 μU/mL)</th>
<th>C-Peptide (0.2 mmol/L)</th>
<th>β-Hydroxybutyrate (2.7 mmol/L)</th>
<th>Sulfonylureas and Other Insulin Secretagogues</th>
<th>Antibody to Insulin</th>
<th>Hypoglycemia Pattern</th>
<th>Diagnostic Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>Below</td>
<td>Below</td>
<td>Below</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>False low</td>
</tr>
<tr>
<td>Yes</td>
<td>Below</td>
<td>Above</td>
<td>Above</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Exogenous insulin</td>
</tr>
<tr>
<td>Yes</td>
<td>Below</td>
<td>Above</td>
<td>Below</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>No</td>
<td>Postprandial</td>
</tr>
<tr>
<td>Yes</td>
<td>Below</td>
<td>Much above</td>
<td>Below</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Postprandial</td>
</tr>
<tr>
<td>Yes</td>
<td>Below</td>
<td>Below</td>
<td>Below</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Postprandial</td>
</tr>
<tr>
<td>Yes</td>
<td>Below</td>
<td>Above</td>
<td>Below</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Postprandial</td>
</tr>
<tr>
<td>Yes</td>
<td>Below</td>
<td>Below</td>
<td>Below</td>
<td>No</td>
<td>No</td>
<td>Yes</td>
<td>No</td>
<td>Autoimmune: antibodies to IR</td>
</tr>
</tbody>
</table>

*Adapted from Ref. 74.*
tracellular α-subunits and a pair of transmembrane β-subunits (disulfide linked) that contain tyrosine kinases.

The IR gene transcript is subject to alternate splicing of exon 11. This exon encodes a 12-amino-acid segment in the C-terminal extension of the receptor’s extracellular α-subunit. Alternative splicing results in the formation of 2 isoforms: IR-A, which lacks this exon 11 sequence, and IR-B, which contains this sequence. IR-A has a high affinity for insulin but a low affinity for IGF-2 (142). Most cells coexpress both isoforms of the IR, but the amount of each varies among different tissues. The IR-A receptor mediates more proliferative effects than IR-B and is expressed predominantly in embryogenesis as well as in certain malignancies (5, 143). The IR-B isoform mediates mostly the metabolic effects of insulin and predominates in metabolic tissues, such as liver, muscle, and adipose tissue (5, 144).

In addition to the IGF-1R and the 2 isoforms of the IR (IR-B and IR-A), both of which are composed of homologous pairs of subunits, there are hybrid receptors; one IGF-1 hemireceptor heterodimerizes with one insulin hemireceptor, either IR-A or IR-B. The hybrids and the homologous receptors are formed in the Golgi during the synthesis of the receptors by random assembly of available hemireceptors. Heterodimerization is believed to occur with an efficiency that is equal to that of homodimerization because of the high homology between the IRs and IGF-1Rs. Therefore, the proportion of hybrids is a function of the mole fraction of each receptor, although unknown factors may influence dimer formation. The mature forms of hybrid receptors bind IGF-1 and IGF-2 with a much greater affinity than insulin (Figure 7) and produce biological effects that are closer to those of typical IGF-1Rs. Under conditions when IRs are downregulated, the ratio of heterodimers to homodimers of IR will increase, amplifying the growth effects. Tissue-specific effects of insulin and the IGFs depend largely on the panel of receptors on the surface of the cell (145–147).

Binding of insulin to the IR is the best described of this group of ligand-receptor interactions. Each insulin molecule has 2 binding sites on the IR, one on each monomer of the receptor dimer. A single insulin molecule binds to 2 α-subunits. The factors involved in differential binding of insulin, IGF-1 and IGF-2 to the two IR isoforms, the IGF-1R, and IGF-1/IR hybrid, are incompletely understood but
appear to depend heavily on subtle differences in the amino acid sequences of the C-domains of IGF-1 and IGF-2. The ability of IGF-2 to signal through the IR and its variants gives IGF-2 broad signaling potential (148, 149).

Binding of IGF-2, IGF-1, or insulin to the extracellular portion of the IGF-1R (or other receptors in this cluster) leads to autophosphorylation of the cytoplasmic tyrosine kinase on the \(\frac{1}{2}\)-subunit of the receptor. This initiates further autophosphorylation of the receptor and the formation of docking sites for several adaptor proteins, ie, IR substrates (IRSs) and sequence homology of collagen (Shc). Activation of the IRS/phosphatidylinositol 3-kinase (PI3K)/protein kinase B (AKT) pathway and the Shc/ERK/MAPK pathway ensues (Figure 7) (5). The ultimate targets of these pathways include members of the E-twenty six and forkhead transcription factor families (150). Although both insulin and IGF-2 bind with high affinity to IR-A, IGF-2 binding leads to more potent activation of the Shc/ERK/MAPK pathway, whereas insulin activates the IRS-1/PI3K/AKT pathway more effectively, leading to differences in transcription factor activation (5, 151, 152).

The consequences of the IGF-2 signaling on glucose homeostasis have been investigated in animals. Current notions are that IGF-2 enhances glucose utilization in a manner similar to insulin, but IGF-2 is much less potent in cells expressing predominantly IR-B. However, IGF-2 circulates at levels that are 100 to 1000 times those of insulin; in theory, in a patient with an IGF-2-oma, IGF-2 through its action on the IR may be fully capable of inducing hypoglycemia (153).

**VIII. IGF-2 Receptor**

IGF-2, in addition to binding to IGF-1Rs and IRs, also binds to the IGF-2 receptor (IGF-2R), more properly the IGF-2/mannose-6-phosphate receptor (154). This receptor contains binding sites for mannose-6-phosphate–containing substrates (that are internalized and delivered to intracellular organelles) and a separate binding site for IGF-2. Most cell surface receptors (eg, IGF-1Rs and IRs) function primarily in intercellular communication. In addition, secondarily, they may serve as a portal for the hormones or other hormone-like messenger molecules into the intracellular pathways, for recycling to the cell surface and for degradation (155–157). In contrast, the IGF-2R’s role has traditionally been to bind IGF-2 at the cell surface, internalize it, and deliver it to the Golgi and then to the lysosomes for degradation (Figure 8) (158–161) without any intercellular signaling function. Recent evidence supports a role of the IGF-2R in cell signaling; when activated by IGF-2, the IGF-2R pro-
motes recruitment and activation of sphingosine kinase, releasing sphingosine 1-phosphate into the extracellular fluid where it binds to a unique sphingosine-recognizing G protein-coupled receptor that activates intracellular signaling events (162).

Like the Igf2 gene, the IGF2R gene is also imprinted, but it is maternally expressed (163). During fetal and neonatal life, it is highly expressed; its expression declines in postnatal life (158). Although certain cancer cells may show loss of imprinting of the Igf2 gene resulting in overexpression of IGF-2 (164–166), loss of heterozygosity of the IGF2R leads to failure of gene expression with potentially impaired IGF-2 clearance. This loss of heterozygosity may account, at least in part, for its associations with certain cancers (167).

IX. IGF Binding Proteins

Although insulin circulates in the unbound form, both IGF-1 and IGF-2 circulate bound to one of the 6 IGFBPs, designated IGFBP-1 to -6 (168, 169). The binding of IGF peptides to IGFBP-1 through -6 is of high affinity (10^{-9} to 10^{-11}) and regulates the biological activity of the IGFs (170). Of the IGFBPs, IGFBP-3 is the most abundant and transports more than 75% of IGF-1 and of IGF-2 in a complex with ALS (171). IGFBP-5 is present...
IGF-2 is usually found in the circulation bound to IGFBP-3. Approximately 70% to 80% of IGF-2 is in a ternary 150-kDa complex containing IGF-2, IGFBP-3, and ALS. Roughly 20% to 30% of IGF-2 forms a 50-kDa binary complex composed of IGFBP-3 and IGF-2, and a small amount circulates as free IGF-2. Circulating free IGF-2 has a half life of 10 to 12 minutes (172). Binding of IGF-2 within the 150-kDa form extends its half-life in the serum to approximately 12 to 16 hours. This complex has limited biological activity in tissues; its large size limits its passage across capillaries. The 50-kDa complex has a shorter half life (20–30 minutes) and is sufficiently small to cross the capillary membrane and gain access to most tissues. The stability of these complexes determines circulating IGF-2 levels (Figure 4) (168, 173–175).

IGFBPs appear to have altered regulation in tumors, with downregulation of IGFBP-3 and upregulation of IGFBP-2 and IGFBP-6 (168, 176–178). IGFBP-6 is an inhibitor of IGF-2, although its role in IGF-2–induced hypoglycemia is unclear (179, 180). Studies suggest that in patients with IGF-2-omas, pro–IGF-2 preferentially forms binary complexes with IGFBP-2 and ternary complexes with IGFBP-5, rather than with IGFBP-3. This may increase the tissue availability of pro–IGF-2, allowing its insulin-like potential to be more fully realized (169).

A group of proteins with structural similarities to the 6 well-established IGFBPs has also been identified. The CCN family (an acronym derived from the first 3 members of the family discovered, CCN1–CCN3) consists of 6 30- to 40-kDa secreted proteins that are extremely cysteine-rich (10% by mass) and contain 4 conserved modular domains with sequence similarities to IGFBPs, von Willebrand factor, thrombospondin, and a cysteine knot (181). CCNs have a binding affinity for IGFs that is 100- to 1000-fold lower than IGFBPs (182, 183). The suggestion by early investigators that the CCN family is part of an IGFBP superfamily has not been substantiated. The relationships between the IGFBPs and CCNs are unclear, but the expression of these proteins is often upregulated in the presence of growth factors. This suggests that these proteins may serve as additional endogenous regulators of IGF action. The role of these proteins in the pathogenesis of cancer is controversial and requires further investigation.
been challenged (183, 184), and the physiological importance of CCN-IGF interactions remains controversial (185, 186).

X. Mechanisms Leading to Hypoglycemia With IGF-2-oma

There are very few direct studies of the effects of IGF-2 and of IGF-2 precursors on glucose metabolism in the whole body. There is much more speculation than evidence, and is largely based on data with insulin action. Insulin-induced hypoglycemia depends primarily on shutting off glucose output from the liver; gluconeogenesis, glycogenolysis, ketogenesis, and the activity of glucose-6-phosphatase are all suppressed (187). Although some part of this process is mediated by insulin’s action on insulin-related receptors on hepatocytes, a substantial part of the shutoff is mediated by insulin binding to its receptors in or near the hypothalamus; hypothalamic neurons signal the liver via the vagus nerve (188–190). IGF-2 is presumed to act in a similar way, but IGF-2 will have relatively greater activity via the IGF-1-related receptors and less via the insulin-type receptors (Figure 6) (191, 192). Although insulin and IGF-2 both have ready access to hepatocytes via fenestrated capillaries, the relative ease of access to the hypothalamus is uncertain.

IGF-2, like insulin, stimulates increased peripheral uptake of glucose by skeletal muscle (193). Free fatty acid levels in the circulation are diminished by IR-mediated inhibition of lipolysis and enhanced esterification in adipocytes (194, 195). Glucose consumption by the tumor contributes little to hypoglycemia, but tumors may up-regulate their hexokinases and glucose transporters (196, 197). Hypoglycemia occurs with fasting but hyperglycemia may occur after feeding (62), similar to patients with insulinomas. Circulating glucagon is suppressed due to the dual action of IGF-2 on the IGF-1R and the IR of pancreatic α-cells. The low GH level is likely due
to negative feedback of elevated IGF-2 via the IGF-1R in cells of the hypothalamus (198, 199). The suppression of glucagon and of GH probably augments the hypoglycemia by impairing the counterregulatory responses to hypoglycemia (1).

In patients with IGF-2-omas, levels of insulin and GH are decreased as are IGF-1 levels, whereas IGF-2 levels may be elevated or within the normal range. After removal of the tumor, IGF-1 levels return to normal, whereas IGF-2 levels fall or remain unchanged (200). It was unclear how hypoglycemia could occur with normal IGF-2 levels. The identification of the incompletely processed pro–IGF-2 (known as big IGF-2) in relative excess and the low concentration of mature IGF-2, suggested that big IGF-2 may be involved (141). IGF-2 mRNA expression is increased in IGF-2-omas, possibly due to loss of imprinting. This loss of imprinting does not appear to be associated with methylation of the imprinting control region, but instead may be related to the activation of abnormal promoters by the tumor (65). The increased mRNA expression may lead to greater quantities of pre-pro–IGF-2, which may exceed the capacity of the processing enzymes and inability to perform normal processing of pro–IGF-2. With IGF-2-omas, pro–IGF-2 is underglycosylated; this may account for the incomplete cleavage of the E-domain that commonly occurs during tumor processing of pro–IGF-2 (201, 202). Removal of the tumor leads to a decrease in the plasma level of big IGF-2, and the bouts of hypoglycemia cease (141, 203).

Animal studies suggest that the hypoglycemic effects of IGF-2 are mediated through the IR. IGF-2 binds to IR-B with an affinity of roughly 5% of that of insulin but binds to IR-A with much higher affinity, 35% to 40% of insulin. (That is multifold greater than the affinity of IGF-1 or proinsulin for the same receptor [204].) Depending on the IR, it is expected that a few-fold higher level of mature IGF-2 or big IGF-2 may well come close to matching the effects of insulin (1).

In addition to the presence of a greater proportion of big IGF-2, another mechanism is suspected to contribute to hypoglycemia when the total IGF-2 level is normal. In individuals with IGF-2-omas, there is impaired formation of the 150-kDa complex, with 80% of the serum IGF-2 present in the 50-kDa complex, compared with 20% to 30% in normal individuals (1, 99, 205). Low levels of ALS in IGF-2-oma patients cause decreased formation of the 150-kDa complex. In addition, the N-linked carbohydrate moiety of IGFBP-3 may interact with the E-domain of big IGF-2, leading to steric interference in the binding of the pro–IGF-2–IGFBP-3 complex to ALS (Figure 4) (169, 206). Free IGF-2 levels also appear to be increased, possibly due to some displacement of normal IGF-2 from the binding proteins by big IGF-2. The levels of free IGF-2 seen in most individuals are sufficiently high to cause hypoglycemia (207). The elevated level of free IGF-2 leads to feedback suppression of GH and then to decreased IGF-1, ALS, and IGFBP-3, which are all GH-dependent (173). Other IGFBPs, namely IGFBP-2, IGFBP-4, and IGFBP-6, are independent of GH, and their expression increases. These binding proteins do not form ternary complexes with IGF-2 and ALS, potentiating the abnormal proportion of 50- to 150-kDa IGF-2–bound complexes (173). The 50-kDa (binary) complexes readily exit the vascular space to produce the hypoglycemic effects of IGF-2 on tissues (208). These hypoglycemic effects involve the direct action of IGF-2 on the IR along with suppression of endogenous insulin secretion from β-cells through its action on the IR and IGF-1R (1). After tumor removal, free IGF-2 levels return to normal as does the proportion of 50- to 150-kDa complexes (173, 203, 207).

**XI. Other Big IGF-2–Linked Syndromes**

Big IGF-2 has also been implicated in another condition known as hepatitis C-associated osteosclerosis. Patients with this syndrome are found to have remarkably increased bone mineral density, which is believed to arise from overstimulation of bone formation by big IGF-2. Similar to patients with IGF-2–linked hypoglycemia, patients with this form of osteosclerosis have increased levels of big IGF-2. However, it appears to be a differently processed isoform of big IGF-2. Interestingly, these patients with osteosclerosis do not develop hypoglycemia, and patients with IGF-2–linked hypoglycemia have not been noted to have increased bone mass. These differences in biological effects of big IGF-2 (also known as IGF-2E) are attributed to differential processing of pre-pro–IGF-2 to form two distinct isoforms: IGF-2E1–104 in the osteosclerosis syndrome and IGF-2E1–88 in IGF-2–linked tumor hypoglycemia (209). Another difference between the two clinical syndromes is that with osteosclerosis, the IGF-2E1–104 isoform retains its ability to form ternary 150-kDa complexes with IGFBP-3 and ALS. (With big IGF-2 from patients with tumor-related hypoglycemia, formation of the 150-kDa binding complex is impaired.) Therefore, no increase in mature unbound IGF-2 is noted in patients with osteosclerosis, and hence hypoglycemia generally does not occur (210). In addition to being carried in a ternary complex, IGF-2E1–104 also circulates bound to IGFBP-2, forming a 50-kDa binary complex. This complex appears to have increased avidity for the bone matrix, where it accumulates and subsequently releases big IGF-2, stimulating new bone formation (210).
XII. Tumor-Directed Therapies

Despite the availability of medical treatments, the mainstay of treatment of hypoglycemia with IGF-2-omas remains surgical resection of the tumor. Multiple case reports have demonstrated an immediate and lasting resolution of symptomatic hypoglycemia after surgical resection of IGF-2-producing tumors. Recurrence of hypoglycemia after surgical resection commonly signals tumor recurrence. When complete resection is not possible, palliative tumor debulking is recommended, as with other hormone-secreting tumors. Debulking in conjunction with tumor embolization has been used with some success. Neoadjuvant therapies such as radiation and chemotherapy have also been reported to abate instances of hypoglycemia, albeit temporarily.

XIII. Hypoglycemia-Directed Therapies

When hypoglycemia persists after surgical interventions have reached their limits, multiple medical modalities have been employed. The most effective agents have been the glucocorticoids. They act to prevent hypoglycemia through several mechanisms: stimulation of hepatic gluconeogenesis, inhibition of peripheral glucose uptake, mobilization of amino acids from extrahepatic sites, and promotion of lipolysis with fatty acid release from adipose tissue. In some patients with hypoglycemia caused by IGF-2-omas, glucocorticoids have been shown to decrease the production of big IGF-2. Because the response to glucocorticoids varies widely among patients, adequate control of clinically meaningful hypoglycemia requires titration of glucocorticoid dose. The effect in preventing tumor-related hypoglycemia is active only as long as glucocorticoid therapy is taken. Hypoglycemia recurs after reduction or cessation of therapy. Recombinant GH (rGH) has been shown to reduce the occurrence of hypoglycemia in patients with IGF-2-producing tumors. In addition to rGH’s stimulatory effect on gluconeogenesis and glycogenolysis, rGH has also been shown to stimulate production of IGFBP-3 and ALS by the liver. It is not clear which mechanism is most responsible for the alleviation of hypoglycemia in these patients. A possible concern with using rGH for long-term treatment of IGF-2-omas, however, is its potential to elevate plasma levels of IGF-1 and of insulin as well as undo the beneficial effects of glucocorticoid therapy on IGF-2 and big IGF-2. There is also a theoretical risk of stimulating growth of the tumor itself. In some patients in whom the benefits of glucocorticoids were inadequate, the addition of rGH has enhanced control of the hypoglycemia.

In general, somatostatin analogs such as octreotide have not been effective in controlling hypoglycemia despite the presence of somatostatin receptors in many IGF-2-omas. In one case, however, the prolonged infusion of somatostatin was associated with a reduction in plasma levels of big IGF-2 associated with an intra-abdominal hemangiopericytoma.

Immediate and short-term correction of hypoglycemia may require parenteral administration of glucose. The long-term appropriateness of such therapy obviously depends on the individual patient’s overall prognosis. Both glucagon, which stimulates hepatic glycogenolysis, and diazoxide, which inhibits pancreatic insulin release, have also been used successfully as short-term treatments for IGF-2-omas.

XIV. Future Therapies

Over the last decades, the role of the IGF system in cancer progression independent of hypoglycemia has been increasingly recognized. Anti-IGF strategies are being investigated as a way of controlling tumor growth and reducing metastatic spread.

A. Tumor-directed therapies (future)

Therapies that are directed at control of cancer growth and reduction of tumor burden should theoretically produce improvement in hypoglycemia caused by IGF-2. One of the pathways by which tumorigenesis is mediated is via the interaction between the IGF-1R and IGF ligands. Inhibition of this pathway might lead to some reduction in hypoglycemia along with control of tumor growth. Some of the strategies include 1) downregulation of IGF-1R, 2) disruption of the receptor-ligand interaction with anti-IGF-1R antibodies, and 3) inhibition of IGF-1R activity with specific kinase inhibitors. These therapies have been shown to slow cancer progression in vitro and in animal studies and are currently being investigated for clinical use in humans.

B. Hypoglycemia-directed therapies (future)

Recall that hypoglycemia in a patient with an IGF-2-oma arises largely from the interaction of big IGF-2 with the IR. Theoretically, inhibition of either the IGF-2 ligand or the IR should prevent hypoglycemia. Inhibition of the IR may be undesirable due to a wide spectrum of metabolic pathways mediated by the receptor. Instead, selective anti-IGF-2 therapy may be a more appropriate goal. Some approaches employ 1) antibodies against IGF-2, 2) up-
regulation of mannose-6-phosphate-IGF-2R as a potential sink for IGF-2, and 3) inhibition of IGF-2 gene transcription.

In one investigational approach, a specific IGF-2 antagonist has been designed that binds IGF-2 with high affinity and traps it. The antagonist is a soluble fusion protein comprised of 1) a modified domain-11 of the IGF-2R (IGF-2R-11) that specifically binds IGF-2 fused to 2) a human IgG Fc domain (235). The affinity for IGF-2 ligand is further enhanced by selectively changing one amino acid in the sequence of the IGF-2R-11. As a result, the novel soluble IGF-2R-11E1534K-Fc protein has high specificity and affinity for IGF-2 in vitro and may find potential future therapeutic applications in vivo. In a similar approach, several human monoclonal antibodies against IGF-2 are being developed. One of those antibodies, IgG1 m610, showed specificity for both pro–IGF-2 and mature IGF-2 without cross-reactivity with insulin or IGF-1 (236).

Another way to decrease bioavailable levels of IGF-2 is to upregulate the endogenous mannose-6-phosphate/IGF-2Rs, which can act as a sink for IGF-2. Recently, it has been proposed that mannose-6-phosphate/IGF-2R is a tumor growth suppressor. In one report, overexpression of this receptor has led to decreased tumor growth and potentially may decrease circulating IGF-2 levels (237).

Another approach is to target big IGF-2 by enhancing its processing into mature IGF-2. Prohormone convertase 4 (PC4) is the enzyme responsible for cleavage of pro–IGF-2 to produce mature IGF-2 (139). Upregulating PC4 may potentially play a role in reducing big IGF-2 levels and thereby ameliorate hypoglycemia. In additional studies, levels of PC4 were found to be reduced in a patient with a giant pleural tumor with hypoglycemia, suggesting that low levels of this converting enzyme might be responsible for impaired processing of IGF-2 (58).

Another proposed strategy to enhance processing of pro–IGF-2 is to create an abzyme (antibody enzyme) that can hydrolyze pro–IGF-2 into mature IGF-2. Abzyme is the name for an antibody or Ig that binds to the target moiety and then hydrolyzes a bond of the antibody-bound molecule. Among the famous abzymes are the pathologic autoantibodies in patients with multiple sclerosis that bind to myelin basic protein and cleave one of its peptide bonds, thereby compromising the protein (238). Abzymes that have high specificity for substrates can be created. When humanized to avoid generating antibodies against themselves, abzymes hold promise as effective therapies. A related mechanism is recalled by the observation that big IGF-2 levels in plasma decrease during storage at −20°C. This raises the possibility that a protease that is a normal component of blood might be harnessed.

Finally, another therapeutic strategy is to interfere with IGF-2 production. Many tumors with IGF-2–linked hypoglycemia appear to over express IGF-2 mRNA with elevated mRNA levels detected in pathology specimens (239, 240). Use of anti–IGF-2 small interfering RNA that inhibits RNA expression has been shown to decrease IGF-2 levels in vitro (241, 242) and might be a promising strategy in the future. A rapidly expanding body of knowledge of the workings of the IGF system will hopefully lead to new therapeutic options for patients with IGF-2–induced tumor hypoglycemia.

**XV. Relevant History**

**A. What’s in a name?**

The IGF-2–secreting benign or malignant tumors in this article can cause fasting hypoglycemia (and in a small subset of patients produce physical changes that overlap with those of acromegaly). It is the secretion of IGF-2 that unites these tumors and is independent of tissue type. In endocrinology, it is a well-established tradition to label a hormone-secreting tumor with the name of its secretory product (and subordinate the histological origin); insulinoma, glucagonoma, gastrinoma, prolactinoma, and vasoactive intestinal peptide-oma are all well established (294).

An obvious advantage of the term IGF-2-oma is that it immediately conveys a lot of information, that it is a tumor and releases clinically significant amounts of IGF-2. The advantages of the new name are magnified by the disadvantages of the old name. NICTH is not a single entity but is a default term introduced to fill a void created in a broad diagnostic niche by the exodus of the islet-cell tumors. Like non-A, non-B hepatitis, it is adynamic, waiting for new knowledge to come along and replace it. The NICTH acronym is obscure; it fails to characterize the condition and is hardly known by physicians outside the field. More importantly, NICTH inadvertently includes other patients with (non–islet-cell) tumors who have hypoglycemia from other (non–IGF-2) causes, eg, medications, hepatic damage, or IGF-1.

The 20th century giant, Robert Graves, poet, classics scholar, and authority on English, supports our right as speakers of English, in contrast to speakers of other languages, to create a new name. He encourages us, noting that “the practice of making new words by declaration is of long standing (295).”
B. Hypoglycemia

The history of hypoglycemia, especially IGF-2 tumor-related hypoglycemia, is rich in surprises. 1) Hypoglycemia as a clinical entity in humans was conceptualized only in the first decade of the 20th century (Table 5), well after the introduction of blood glucose measurements. 2) Hypoglycemia with islet-cell tumors was recognized in the 1920s by analogy to hypoglycemic episodes in patients with diabetes who were receiving insulin, the new miracle drug. 4) Hypoglycemia with nonpancreatic tumors, first mentioned in 1929, lagged behind the islet-cell tumors and was conceptually fragmented and misattributed.

C. Major IGF-2-oma landmarks

1) The insulin radioimmunoassay demonstrated that hypoglycemia with islet-cell tumors was associated with insulin excess, whereas that from non–islet-cell tumors was not (243). 2) At the National Institutes of Health (NIH), Megyesi, Roth, Kahn, Neville, and Gorden, with partially purified preparations, introduced a novel radio-receptor assay that demonstrated IGF-related material in the blood and tumors of patients with non–islet-cell tumors and its disappearance after successful surgery (203, 244–251, 296, 297). The partially purified preparations, then known collectively as nonsuppressible insulin-like activity soluble (NSILA-s) contained both IGF-1 and IGF-2 (Figure 9). 3) IGF-2 and IGF-1 were purified (252–254). 4) That group and others recognized big IGF-2, an incompletely processed precursor of IGF-2 that is biologically active but binds poorly to IGFBPs.

D. Pioneer studies

The emergence of IGF-2 and its links to tumor hypoglycemia are described here in parallel with the scientific advances of the times (Table 5). In the 19th century, experimental surgery by Bernard, Minkowski, von Mering (255), and others implicated the brain, liver, and pancreas in glucose homeostasis. Minkowski and von Mering’s experiments on pancreatectomized dogs demonstrated that the pancreas was the source of an antidiabetic internal secretion and led others to conclude that the islets of Langhans discovered in 1869 were the source. After a 3-decade worldwide search by scores of researchers, insulin was ultimately isolated in Toronto in 1922. Internal secretion, conceptualized by Bernard, was replaced by hormone, the term introduced by Starling in 1905 (256).

E. Emergence of hypoglycemia

Measurements of glucose in urine of patients with diabetes by cupric oxide date to the 1840s. Reliable blood glucose measurements were introduced by Pavy in the early 1900s (257, 258). The first patients reported to have hypoglycemia suffered from Addison’s disease (259), pituitary destruction by an adenoma (260), and terminal starvation as part of the treatment for diabetes in the pre-insulin era (261), decades after Minkowski observed hypoglycemia in animals after hepatectomy (262). The symptoms and signs of hypoglycemia in humans were unappreciated until the introduction of insulin therapy in early 1920s. An examination of patient records from 1915 at a major New York hospital revealed that urine glucose

<table>
<thead>
<tr>
<th>Year</th>
<th>Finding</th>
<th>Scientist(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1886</td>
<td>Fatal hypoglycemia in animals after hepatectomy</td>
<td>Minkowski (262)</td>
</tr>
<tr>
<td>1910</td>
<td>Hypoglycemia in Addison’s disease</td>
<td>Porges (259)</td>
</tr>
<tr>
<td>1912</td>
<td>Hypoglycemia in pituitary adenoma</td>
<td>Cushing (260)</td>
</tr>
<tr>
<td>1920</td>
<td>Hypoglycemia in emaciated diabeticsb</td>
<td>Joslin (261)</td>
</tr>
<tr>
<td>1921</td>
<td>Signs of hypoglycemia in animals after hepatectomy</td>
<td>Mann and Magath (263)</td>
</tr>
<tr>
<td>1922</td>
<td>Hypoglycemia with overdoses of insulin in pancreatectomized dogs and patients with diabetes</td>
<td>Banting et al (289)</td>
</tr>
<tr>
<td>1924</td>
<td>Concept of spontaneous hyperinsulinism</td>
<td>Harris et al (265)</td>
</tr>
<tr>
<td>1927</td>
<td>Hypoglycemia in islet cell tumors, insulinoma</td>
<td>Wilder et al (266)</td>
</tr>
<tr>
<td>1928</td>
<td>Hypoglycemia in glycogen storage diseases</td>
<td>Snapper and van Creveld (290)</td>
</tr>
<tr>
<td>1929</td>
<td>Extrapancreatic tumors associated with hypoglycemia</td>
<td>Nadler and Wolf (2)</td>
</tr>
<tr>
<td>1933</td>
<td>Insulin shock treatment in psychiatric patients</td>
<td>Sakel (264)</td>
</tr>
<tr>
<td>1938</td>
<td>Triad necessary to diagnose hypoglycemia</td>
<td>Whipple (86)</td>
</tr>
<tr>
<td>1960</td>
<td>Radioimmunoassay for insulin</td>
<td>Yalow and Berson (243)</td>
</tr>
<tr>
<td>1974</td>
<td>Receptor assay implicates IGFs</td>
<td>Megyesi et al (245)</td>
</tr>
<tr>
<td>1978</td>
<td>IGF-1, IGF-2 isolated and sequenced</td>
<td>Rinderknecht and Humbel (253, 254)c</td>
</tr>
<tr>
<td>1981</td>
<td>High molecular weight IGF-like material detected in plasma</td>
<td>Gorden et al (203)</td>
</tr>
<tr>
<td>1988</td>
<td>Big IGF-2 recognized</td>
<td>Daughaday et al (141), Grunberger et al (83)</td>
</tr>
</tbody>
</table>

a Adapted from Ref. 260 and revised.

b Before the introduction of insulin therapy, severe hyperglycemia in patients with diabetes was sometimes treated by medically supervised starvation.

c In their original paper, Rinderknecht and Humbel (254) used the Roman numerals (I and II) to refer to the two different IGF molecules. This terminology was slowly replaced by Arabic numerals (1 and 2). With computer typography, the Arabic numerals are better distinguished.

814 Dynkevich et al Tumors, IGF-2, and Hypoglycemia Endocrine Reviews, December 2013, 34(6):798–826

Downloaded from https://academic.oup.com/edrv/article/34/6/798/2354658 by guest on 21 July 2024
measurements were the major tool for screening as well as diagnosis and follow-up of patients with diabetes mellitus. For patients with glycosuria, one measurement of blood glucose was carried out to exclude the diagnosis of renal glycosuria.

F. Emergence of the signs of hypoglycemia

The progression from muscle weakness to flaccid paralysis, convulsions, and death was first observed in hepatectomized geese (262). These signs were ameliorated by iv glucose (263). The first patients treated with insulin (1922) brought hypoglycemia to the forefront of clinical medicine. Inexperience with this new drug, variability in potency of the early batches, and the rarity of blood glucose monitoring frequently resulted in hypoglycemia. Soon, the signs and symptoms of hypoglycemia expanded to include visual disturbances and perspiration as well as cognitive and mental changes.

The most extensive data on severe hypoglycemia in humans (including seizures and coma) was garnered during the 3 decades of insulin shock therapy for the treatment of schizophrenia (264). (Typically 100–150 U insulin were given to produce coma and seizures.) Because of serious side effects including deaths, insulin was replaced by neuroleptic drugs and electroconvulsive therapy.

Using the analogy of hyperthyroidism and hypothyroidism, Harris (265) postulated the existence of hypoglycemia caused by insulin overproduction by islet-cell tumors as a mirror image of type 1 diabetes. On review, it is uncertain whether any of his 5 reported patients had an insulinoma. The concept of hyperinsulinism was strengthened by a 1927 report of a patient with an islet-cell carcinoma metastatic to the liver (266) and a 1929 report of severe hypoglycemia in a patient with an islet-cell tumor of the pancreas that was cured by surgery (267).

In the ensuing decades, hundreds of cases of islet-cell tumors with hypoglycemia helped define the clinical spectrum. However, IGF-2-omas and other non–islet-cell tumors with hypoglycemia had a slow emergence. The first non-islet tumors (presumably IGF-2-omas) associated with hypoglycemia were hepatocellular carcinomas (2, 3). In 1930, removal of a large fibrosarcoma from the mediastinum relieved the patient of symptoms of hypoglycemia (268). Recurrence of the tumor with the hypoglycemia confirmed the relationship (269). In the same year, a patient was reported with fatal hypoglycemia associated with an adrenal tumor (270). Major reviews of tumor-induced hypoglycemia or of hypoglycemia more broadly are surprisingly deficient in coverage of IGF-2-omas and other non–islet-cell tumor hypoglycemia entities (87, 271, 272). The slow emergence of the syndrome is ascribed to 1) the wide diversity of tissues involved and 2) that hypoglycemia can occur with destruction of liver or adrenal parenchyma due to any pathology including tumors.

G. Plasma insulin measurements before 1960

Before 1960 (ie, before radioimmunoassays), insulin was measured solely by bioassay, in vivo and later in vitro, and referred to as insulin-like activity (ILA). Using the diaphragm muscle and epididymal fat pad (isolated adipocytes were introduced later), good results were obtained with pure insulin and pancreatic extracts, but plasma and plasma extracts gave results that were difficult to interpret. In contrast to pure insulin, whose bioactivity was neutralized, or suppressed, by anti-insulin antibodies, most of the serum ILA was unaffected (ie, nonsuppressible) by anti-insulin antibody, hence the designation NSILA for this part of the total ILA. Much of the bioactivity was of high molecular weight, releasable at low pH or with other manipulations. Other designations for this activity were bound insulin and atypical insulin. Only with the heroic isolation, purification from plasma, and sequencing of IGF-1 and IGF-2 in 1976 (254, 273), and the
recognition of the role of IGFBPs, was the nature of insulin-like bioactivities of plasma made more clear. Interestingly, despite the similarity of the amino acid sequences of the IGFs to that of insulin and overlapping reactivity with receptors, insulin and the IGFs are recognized only by homologous antisera and are unreactive with heterologous antibodies.

H. Radioimmunoassay of insulin

The introduction (243) of the radioimmunoassay for insulin (which detected insulin, proinsulin, and its intermediates but not IGF-1 or IGF-2) made it clear that insulinomas release insulin inappropriately, whereas non-islet-cell tumors do not. The radioimmunoassay of the 1960s had less sensitivity and precision than the insulin assays of today. The assay was not sensitive enough to include those patients with insulinomas with levels of insulin that are not elevated in an absolute sense but are high in the context of hypoglycemia. With insulin levels equivalent to basal levels or below, the amount and existence of insulin in the sample was always a little uncertain. We attribute to these technical problems the occasional reports of insulin production by non-islet-cell tumors. Such reports ceased after improvements of the radioimmunoassay (243, 274).

I. Extant theories before 1974

Many theories were propounded to explain the mechanism by which large non-islet-cell tumors produce hypoglycemia (187, 275–280). Unger (277) in 1966 clearly summarized the scientific community’s collective wisdom. Overutilization of glucose by the tumor was a widely held theory, based on classic observations that tumors metabolize large amounts of glucose. A corollary was that the tumors outstrip the ability of the liver to compensate. Unger (277) marshaled data to counter this notion and postulated that the liver is very likely able to meet the demands of even the most voracious tumors. Unger (277) pointed instead to evidence of deficient counterregulation: “deficient exclusion of glucose from peripheral tissues” and “deficient compensatory glucose production by liver” at the same time that the liver has adequate stores of glycogen (that can be mobilized by exogenous administration of glycogenolytic hormones). He proposed humoral mechanisms linked to an insulin action, but the molecules responsible were as yet undefined. Eastman et al (78) in 1992 verified that glucose consumption by these types of tumors was modest, whereas peripheral (muscle) utilization of glucose was high.

J. Megyesi, NIH, and the 1974 Watershed

From 1974 to 1981, with a new radioreceptor assay, Megyesi et al (245) at the NIH published a series of studies that linked the hypoglycemia in patients with non–islet-cell tumors to IGFs, then known as NSILA-s. Using partially purified peptides prepared by Humbel and Rinderknecht (253), Megyesi et al (245) developed a reasonably sensitive and specific radioreceptor assay with cell membranes from rat liver, which is now known to recognize mostly IGF-2. The results were extensively verified as more highly purified IGF preparations became available, including purified IGF-2. Elevated levels of this material were demonstrated in blood from some patients with non-islet-cell tumors (but not elevated in patients with insulinomas), and plasma levels fell dramatically with successful surgery. Overall, 19 of 52 patients studied with different tumors had elevated NSILA-s levels; in addition to an etiology, these studies provided a previously unrecognized unity to the multiple groups of tumor hypoglycemia entities (203). These studies also showed a higher molecular weight form of this material in blood from patients with non–islet-cell tumors (but not in others), likely corresponding in retrospect to big IGF-2. By introducing new extraction procedures, they also led the way in detecting IGF-2 in the tumors (251).

In the late 1970s when IGF-1 could be clearly distinguished from IGF-2 by more specific assays (282), the role of IGF-2 in tumor hypoglycemia became more widely accepted, although it has persisted as a subject of debate. Whereas some investigators such as Daughaday et al (283) reported that IGF-2 concentrations were increased in 10 of 14 serum samples from patients with non–islet-cell tumors and hypoglycemia, others, including Widmer et al (200), could not demonstrate similar increases in IGF-2 in samples from similar patients with non–islet-cell tumors. Possible causes included extraction methods that yielded poor recovery of IGF-2 material (251) or lability of IGF-2 material in stored samples, as described by Merimee (284). IGF-2 levels when analyzed 8 to 15 weeks after collection, appeared to be significantly diminished compared with samples analyzed at the time of collection. The most important observation came from Daughaday in 1988, when he described a woman with leiomyosarcoma and hypoglycemia (141), who despite having normal levels of IGF-2, had elevated levels of big IGF-2. He also found big IGF-2 in the tumor itself and demonstrated that levels of serum big IGF-2 dropped nearly to zero after the removal of the tumor. Big IGF-2 can suppress GH secretion and thereby reduce IGF-1 levels.

Later, IGFBPs were described and observed to be suppressed by IGF-2, further enhancing bioactivity of IGF-2 and contributing to hypoglycemia. Further advances led
to mapping of the IGF-2 gene and sequencing of big IGF-2 proteins (253, 254).

Acknowledgments

We recall contributions by many colleagues including C. Ronald Kahn, David M. Neville, Jr., Phillip Gorden, and Maxine A. Lesniak, but especially Klára Megyesi who in 1974 to 1978 at NIH in NIDDK’s Diabetes Branch performed (without salary) trailblazing experiments that implicated IGF-2–related molecules as mediators of hypoglycemia in patients with non-islet–cell tumors. The memorial note by Zsuzsa Beres, Klára’s daughter (excerpted and edited here), shows how Klára Megyesi’s life (1928–2004) reflects the tragedies and miracles of 20th century Europe. Born in secrecy on June 13, 1928, to a young unwed daughter of a prosperous Jewish family in Budapest, Klára was relinquished to a state institution. At age 2, Klára was adopted by a poor Jewish couple who raised and loved her; they were deported by the Nazis to Auschwitz in 1944, never to return. Klára, on a forced march from Budapest to Vienna, was rescued by the Swedish diplomat Raoul Wallenberg. In 1948, Klára Megyesi began a lifelong association with Semmelweis Medical University, especially its First Department of Internal Medicine, with board certification (1957) and the PhD (1971) covering work on diabetes-related liver disorders (including some work with Prof Sheila Sherlock in London). Klára’s seminal NIH work led to associate professorship (1978) and a new thesis (1979) for the postdoctoral degree, Doctor of Medical Science (1979); she was the first woman in internal medicine in Hungary to gain this degree. Klára was also revered as a teacher and as a physician who cared deeply about her patients. She worked until the day of her tragic accidental death on March 4, 2004.

Address requests for reprints to: Dr Jesse Roth, MD, FACP, Investigator, Feinstein Institute for Medical Research, Laboratory of Diabetes and Diabetes-Related Research, 330 Community Drive, Manhasset, NY 11030. E-mail: jesserorhmd@hotmail.com.

This work was funded by the Feinstein Institute for Medical Research, Manhasset, New York.

Disclosure Summary: The authors have nothing to disclose.

References

22. Ikeda K, Mizuguchi M, Yoshida H, et al. Preclinical Cush- ing’s syndrome associated with non-islet cell tumor hypo-


38. de Boer J, Jager PL, Wiggers T, et al. The therapeutic chal-


117. Lighten AD, Hardy K, Winston RM, Moore GE. IGF2 is
152. Nissley P, Kiess W, Sklar M. The insulin-like growth factor...


185. Grotendorst GR, Lau LF, Perbal B. CCN proteins are distinct from and should not be considered members of the insulin-like growth factor-binding protein superfamily. Endocrinology. 2000;141:2254–2256.


211. Teale JD, Wark G. The effectiveness of different treatment options for non-islet cell tumour hypoglycemia. *Clin Endocrinol (Oxf).* 2004;60:457–460.


288. Banting FG, Best CH, Collip JB, Campbell WR, Fletcher AA, Macleod JIR, Noble EC. The effect produced on di-


