

of expression of *Casr* in GPC was lethal; affected embryos died before day 13 of embryonic life. Development of a mouse model in which “knock-down” of *Casr* expression in GPC at day 16 to 17 of embryonic life after treatment with an estrogen receptor agonist (tamoxifen) resulted in offspring with modestly decreased growth of long bones despite expansion of the hypertrophic zone of the growth plate, decreased differentiation to terminal chondrocytes, and decreased expression of *Igf1* and *Igf1r* by GPC.

The authors concluded that: (1) the elimination of a functional CaSR in PTC also depressed *Casr* expression in osteoblasts (hypothetically through hypercalcemia and increased signaling by the PTH receptor in bone); (2) the CaSR was innately essential for osteoblast differentiation, function, and survival; and (3) that partial and delayed loss of the CaSR in hypertrophic chondrocytes reduced chondrocyte differentiation in part through decreased IGF-1R signaling.

Chang W, Tu C, Chen TH, Bikle D, Shoback D. The extracellular calcium-sensing receptor (CaSR) is a critical modulator of skeletal development. *Sci Signa*. 2008;1: ra1\ [DOI:10.1126/scisignal.1159945]

Editor's Comment: *This research has demonstrated the individual importance of the CaSR in PTCs, OBs, and hypertrophic chondrocytes.³ Interestingly, “knock-out” of*

the CaSR in PTCs secondarily impaired expression of Casr in osteoblasts, demonstrating clearly the interdependence of the parathyroid-osteoblast axis. A study of the effect of overexpression of Casr in PTCs upon OB expression of Casr and bone morphology would be of interest. This manuscript also introduced a new feature of the electronic journal—Science Signaling—sponsored by the AAAS, that has until now published review articles and didactic materials on the subjects of intra- and intercellular communications.⁴ It will now publish original research articles as well.

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Insulin Analogues...and Cancer?

Insulin analogues with different pharmacokinetics were created by inserting point modifications into the amino acid sequence of human insulin, particularly the C-terminus of its beta-chain which is not involved in binding to the insulin receptor (IR). However, these modifications may alter binding affinity for the closely related type 1 insulin-like growth factor (IGF) receptor (IGF1R). Thus, Weinstein et al asked the important question of how these analogues compare to insulin and IGF-I in eliciting IGF-I activities (namely, proliferation and protection from serum starvation-induced apoptosis) in cultured cancer cells.

They studied 2 long-acting insulin analogues (glargine [Lantus®] and detemir [Levemir®]) and 2 short-acting analogues (lispro [Humalog®] and aspart [Novolog®]) in 3 different cell lines: HCT-116 colorectal, PC-3 prostate and MCF-7 breast cancer cells. All experiments were conducted in vitro. Results are summarized in the Table.

HCT-116 cells showed a dose-dependent proliferative response to both glargine and detemir at 72 hours, but not IGF-I (all doses about +21%) nor insulin (all doses negligible effect). The authors then turned to signaling pathways that may underlie the hormonal effects in HCT-116 cells. Basal expression levels of IR and IGF1R were equivalent when measured by Western immunoblotting and immunofluorescent staining. After

Summary of effects on cell behavior in vitro.

Effect	Insulin	Glargine	Detemir	Lispro	Aspart	IGF-I
HCT-116 proliferation at 96 hrs (compared to untreated cells)	+0.4%	+22%	+17%	-	-	+24%
HCT-116 proliferation at 48 hrs ¹ (compared to untreated cells)	+7%	-	-	+20%	0	+22%
PC-3 proliferation at 72 hrs (compared to untreated cells)	+2%	+17%	+15%	-	-	+25%
MCF-7 proliferation at 72 hrs (compared to untreated cells)	0	+14%	+6%	-	-	+22%
HCT-116 % apoptotic cells at 12 hrs (control = 23%)	25%	15%	18%	-	-	17%
HCT-116 % apoptotic cells at 24 hrs (control = 30%)	30%	26%	25%	-	-	24%

¹ by MMT assay; all other proliferation experiments were measured by cell counts. Apoptotic cells were quantified via flow cytometry of Annexin V-FITC and Propidium Iodide labeled cells.

10 and 20 minutes of treatment, glargine phosphorylated both IR and IGF1R, and detemir phosphorylated IR but not IGF1R. Glargine further led to increased phosphorylation of both Akt and ERK, representing the 2 major signaling cascades of IR and IGF1R, without changes in the total protein amounts; phosphorylation was maximal at 20 minutes and decreased by 60 minutes. In a test of relative potencies, cells were treated for 30 minutes with each hormone at 50 ng/mL. Glargine and insulin both significantly increased the amount of phosphorylated Akt in comparison to untreated cells, while detemir and IGF-I did not significantly alter Akt phosphorylation. Insulin alone significantly increased ERK phosphorylation.

The authors concluded that at the supra-physiologic doses tested, glargine and detemir have significant IGF-I-like mitogenic activity, which is not shared by insulin. The authors' warning bears repeating: current evidence shows that neither IGF-I nor insulin (and hence, one would expect the insulin analogues as well) can cause malignant transformation. However, IGF-I does increase the aggressivity of already transformed cells. Thus,

the question raised by this paper is whether long-term exposure to the insulin analogues can likewise affect cancer behavior.

Weinstein D, Simon M, Yehezkel E, Laron Z, Werner H. Insulin Analogues Display IGF-I-like mitogenic and anti-apoptotic activities in cultured cancer cells. *Diabetes Metab Res Rev.* 2009; 25:41-49.

Editor's Comment: *It would take a colossal leap to answer the underlying question based on the data of this pilot study. However, the results are intriguing enough to suggest more rigorous investigations are warranted. The high prevalence of both cancer and diabetes in our society, plus the widespread long-term use of these modified insulin analogues, makes the question an important one to answer. If—and this is a big if—it pans out that one or more of the insulin analogues is more stimulatory for cancer behavior, then cancer risk will become yet another factor clinicians must consider in selecting the particular insulin regimen for an individual patient.*

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Continuous Glucose Monitoring and Intensive Treatment of Type 1 Diabetes

The Juvenile Diabetes Research Foundation (JDRF) Continuous Glucose Monitoring Study Group reported their findings of a multicenter clinical trial which randomly assigned 322 adults and children with type 1 diabetes to continuous glucose monitoring (CGM) or a control group which performed blood glucose monitoring with a glucose meter. All subjects were followed for 6 months to determine whether CGM helped to produce a sustained lowering of HbA1c and a reduction in hypoglycemia. The subjects were stratified by age: 8 to 14 years, 15 to 24 years, and over 25 years of age, and by HbA1c $\leq 8\%$ and $> 8\%$. Individuals with HbA1c of $< 7\%$ or $> 10\%$ were excluded. Subjects had to be using an insulin infusion pump, or at least 3 daily insulin injections, to control their diabetes and could not have had experience with CGM for the 6 months prior to the trial. The final study group included subjects who used either the Dexcom 7® (Dexcom™), the Mini-Med Paradigm® Real Time Insulin Pump and Continuous Glucose Monitoring System (Medtronic), or the FreeStyle Navigator® (Abbott Diabetes Care) according to the manufacturer's instructions which included specific calibration procedures and replacement of the sensors every 3 to 7 days.

Subjects were instructed to verify the accuracy of CGM determinations with self blood glucose meters before making treatment decisions. Subjects were also given written instructions on how to use the data generated by the CGM and blood glucose meters to make real-time adjustments in insulin doses. Target pre-meal blood glucose values were identical for the study group and the control group, 70 to 130 mg/dL (3.9

to 7.2 mmol/L); target peak post-prandial values were < 180 mg/dL (10 mmol/L), and bedtime overnight values 100 to 150 mg/dL (5.6 to 8.3 mmol/L). Subjects were seen at weeks 1, 4, 8, 13, 19 and 26 with one telephone contact between each visit to review glucose data and adjust diabetes management. After visits at 13 and 26 weeks, the control group used a blinded CGM for one week in order to compare continuous glucose profiles with the treated group. HbA1c was measured at 13 and 26 weeks and adverse events including severe hypoglycemia (defined as requiring assistance from another person and/or the use of glucagon), hyperglycemia with ketoacidosis, or other events were recorded.

The trial included 322 subjects (CGM group $n=165$; control group $n=157$); 114 patients were between 8 to 14 years of age (CGM group $n=56$, control group $n=58$), 100 subjects between 15 to 24 years of age (CGM group $n=57$, control group $n=53$) and 98 participants were over 25 years of age (CGM group $n=52$, control group $n=46$). A significant between group difference in the change in HbA1c from baseline to 26 weeks was seen in subjects who were 25 years of age or older, but not in those 15 to 24 years of age, or 8 to 14 years of age. In addition, in the CGM group over 25 years of age there were improvements in all measures of glycemic control including pre-meal and post peak-meal glucose values. The secondary analysis showed more patients in the CGM group had a reduction of 10% or more in mean HbA1c and more patients achieved their target HbA1c of $< 7.0\%$. Among subjects 15 to 24 years of age, the mean decrease in HbA1c from baseline to