

The following abstracts from The Endocrine Society Journals have been selected by the editors as being particularly relevant to readers interested in translational science.

Minireview: Hormonal and Metabolic Mechanisms of Diabetes Remission after Gastrointestinal Surgery

Joshua P. Thaler and David E. Cummings

(*Endocrinology*, published April 16, 2009, 10.1210/en.2009-0367)

ABSTRACT

Bariatric surgery is the most effective available treatment for obesity. The most frequently performed operation, Roux-en-Y gastric bypass (RYGB), causes profound weight loss and ameliorates obesity-related comorbid conditions, especially type 2 diabetes mellitus (T2DM). Approximately 84% of diabetic patients experience complete remission of T2DM after undergoing RYGB, often before significant weight reduction. The rapid time course and disproportional degree of T2DM improvement after RYGB compared with equivalent weight loss from other interventions suggest surgery-specific, weight-independent effects on glucose homeostasis. Potential mechanisms underlying the direct antidiabetes impact of RYGB include enhanced nutrient stimulation of lower intestinal hormones (e.g. glucagon-like peptide-1), altered physiology from excluding ingested nutrients from the upper intestine, compromised ghrelin secretion, modulations of intestinal nutrient sensing and regulation of insulin sensitivity, and other changes yet to be fully characterized. Research aimed at determining the relative importance of these effects and identifying additional mechanisms promises not only to improve surgical design but also to identify novel targets for diabetes medications.

Minireview: Endocannabinoids and Their Receptors as Targets for Obesity Therapy

Annette D. De Kloet and Stephen C. Woods

(*Endocrinology*, published April 16, 2009, 10.1210/en.2009-0046)

ABSTRACT

As the incidence of obesity continues to increase, the development of effective therapies is a high priority. The endocannabinoid system has emerged as an important influence on the regulation of energy homeostasis. The endocannabinoids anandamide and 2-arachidonoylglycerol act on cannabinoid receptor-1 (CB1) in the brain and many peripheral tissues causing a net anabolic action. This includes increasing food intake, and causing increased lipogenesis and fat storage in adipose tissue and liver. The endocannabinoid system is hyperactive in obese humans and animals, and treating them with CB1 antagonists causes weight loss and improved lipid and glucose profiles. Although clinical trials with CB1 antagonists have yielded beneficial metabolic effects, concerns about negative affect have limited the therapeutic potential of the first class of CB1 antagonists available.

Pegylated Leptin Antagonist Is a Potent Orexigenic Agent: Preparation and Mechanism of Activity

Eran Elinav, Leonora Niv-Spector, Meirav Katz, Tulin O. Price, Mohammed Ali, Michal Yacobovitz, Gili Solomon, Shay Reicher, Jessica L. Lynch, Zamir Halpern, William A. Banks, and Arie Gertler

(*Endocrinology*, published April 2, 2009, 10.1210/en.2008-1706)

ABSTRACT

Leptin, a pleiotropic adipokine, is a central regulator of appetite and weight and a key immunomodulatory protein. Although inborn leptin deficiency causes weight gain, it is unclear whether induced leptin deficiency in adult wild-type animals would be orexigenic. Previous work with a potent competitive leptin antagonist did not induce a true metabolic state of leptin deficiency in mice because of a short circulating half-life. In this study, we increased the half-life of the leptin antagonist by pegylation, which resulted in significantly increased bioavailability and retaining of antagonistic activity. Mice administered the pegylated antagonist showed a rapid and dramatic increase in food intake with weight gain. Resulting fat was confined to the mesenteric region with no accumulation in the liver. Serum cholesterol, triglyceride, and hepatic aminotransferases remained unaffected. Weight changes were reversible on cessation of leptin antagonist treatment. The mechanism of severe central leptin deficiency was found to be primarily caused by blockade of transport of circulating leptin across the blood-brain barrier with antagonisms at the arcuate nucleus playing a more minor role. Altogether we introduce a novel compound that induces central and peripheral leptin deficiency. This compound should be useful in exploring the involvement of leptin in metabolic and immune processes and could serve as a therapeutic for the treatment of cachexia.

Association between Serum Insulin-Like Growth Factor (IGF) I and IGF Binding Protein-3 and Lung Function

Sven Gläser, Nele Friedrich, Ralf Ewert, Christoph Schäper, Matthias Nauck, Marcus Dörr, Henry Völzke, Stephan B. Felix, Alexander Krebs, Henri Wallaschofski, and Beate Koch

(*J Clin Endocrinol Metab*, published April 28, 2009, 10.1210/jc.2008-2662)

ABSTRACT

Background: There is strong evidence that IGF-I and IGF binding protein 3 (IGFBP-3), as central mediators of endocrine and finally metabolic or anabolic effects of GH, were associated with increased lung size in acromegaly or a decrease of respiratory muscle pressures in patients with GH deficiency. The aim of the present study was to further clarify the impact of IGF-I and IGFBP-3 levels on lung volumes and respiratory pressures in a general adult population.

Material and Methods: From the Study of Health in Pomerania, 1326 subjects aged 25 to 85 yr participated in standardized pulmonary function testing. IGF-I and IGFBP-3 levels were measured with the Immulite 2500 system. Multivariable linear regression analyses adjusted for age, sex, body mass index, physical activity, and smoking were performed.

Results: In men, positive linear associations between IGF-I and IGF-I/IGFBP-3 ratio with forced expiratory volume in 1 sec (FEV1) as well as with forced vital capacity (FVC) were detected across all ages, whereas in women this positive association was only detectable above 50 yr. Furthermore, the analyses indicated positive linear relations of IGF-I/IGFBP-3 ratio with FEV1 and FVC, respectively. No significant relations between IGF-I or IGFBP-3 and maximal inspiratory pressure was detectable in both sexes.

Conclusion: In conclusion, higher IGF-I levels were associated with higher lung volumes in men, whereas in women this association was only detectable in subjects older than 50 yr. Higher IGF-I values were not associated with increased respiratory muscle strength measured as maximal inspiratory pressure.

Modulation of Gonadotropin-Releasing Hormone Pulse Generator Sensitivity to Progesterone Inhibition in Hyperandrogenic Adolescent Girls—Implications for Regulation of Pubertal Maturation

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(J Clin Endocrinol Metab, published April 7, 2009, 10.1210/jc.2008-2606)

ABSTRACT

Context: Adult women with polycystic ovary syndrome (PCOS) have decreased GnRH pulse generator sensitivity to progesterone (P)-mediated slowing. This defect is androgen mediated because it is reversed with androgen receptor blockade. Adolescent hyperandrogenism often precedes PCOS.

Objective: The aim of the study was to evaluate GnRH pulse generator sensitivity to P-mediated slowing in normal and hyperandrogenic girls.

Design: We conducted a controlled interventional study.

Setting: The study was conducted in a general clinical research center.

Participants: A total of 26 normal control (NC) and 26 hyperandrogenic (HA) girls were studied.

Intervention: Frequent blood sampling was performed for 11 h to assess LH pulse frequency before and after 7 d of oral estradiol and P.

Main Outcome Measure: We measured the slope of the percentage reduction in LH pulse frequency as a function of d 7 P (slope).

Results: Overall, Tanner 3-5 HA subjects were less sensitive to P-mediated slowing than Tanner 3-5 NC (slope, 4.7 ± 3.4 vs. 10.3 ± 7.7 ; $P = 0.006$). However, there was variability in the responses of HA subjects; 15 had P sensitivities within the range seen in NC, whereas nine were relatively P insensitive. The two groups had similar testosterone levels. Fasting insulin levels were higher in P-insensitive HA girls (39.6 ± 30.6 vs. 22.2 ± 13.9 mIU/ml; $P = 0.02$), and there was an inverse relationship between fasting insulin and P sensitivity in HA girls ($P = 0.02$). Tanner 1-2 NC had lower testosterone levels and were more P sensitive than Tanner 3-5 NC (slope, 19.3 ± 5.8 ; $P = 0.04$).

Conclusions: Hyperandrogenism is variably associated with reduced GnRH pulse generator sensitivity to P-mediated slowing during adolescence. In addition to androgen levels, insulin resistance may modulate P sensitivity.

Lower Testosterone Levels Predict Incident Stroke and Transient Ischemic Attack in Older Men

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(J Clin Endocrinol Metab, published April 7, 2009, 10.1210/jc.2008-2416)

ABSTRACT

Context: Lower circulating testosterone concentrations are associated with metabolic syndrome, type 2 diabetes, carotid intima-media thickness, and aortic and lower limb arterial disease in men. However, it is unclear whether lower testosterone levels predict major cardiovascular events.

Objective: We examined whether lower serum testosterone was an independently significant risk factor for symptomatic cerebrovascular events in older men.

Design: This was a prospective observational study with median follow-up of 3.5 yr.

Setting: Community-dwelling, stroke-free older men were studied.

Participants: A total of 3443 men at least 70 yr of age participated in the study.

Main Outcome Measures: Baseline serum total testosterone, SHBG, and LH were assayed. Free testosterone was calculated using mass action equations. Incident stroke or transient ischemic attack (TIA) was recorded.

Results: A first stroke or TIA occurred in 119 men (3.5%). Total and free testosterone concentrations in the lowest quartiles (<11.7 nmol/liter and <222 pmol/liter) were associated with reduced event-free survival ($P = 0.014$ and $P = 0.01$, respectively). After adjustment including age, waist-hip ratio, waist circumference, smoking, hypertension, dyslipidemia, and medical comorbidity, lower total testosterone predicted increased incidence of stroke or TIA (hazard ratio = 1.99; 95% confidence interval, 1.33–2.99). Lower free testosterone was also associated (hazard ratio = 1.69; 95% confidence interval, 1.15–2.48), whereas SHBG and LH were not independently associated with incident stroke or TIA.

Conclusions: In older men, lower total testosterone levels predict increased incidence of stroke or TIA after adjusting for conventional risk factors for cardiovascular disease. Men with low-normal testosterone levels had increased risk. Further studies are warranted to determine whether interventions that raise circulating testosterone levels might prevent cerebrovascular disease in men.

Activated Ask1-MKK4-p38MAPK/JNK Stress Signaling Pathway in Human Omental Fat Tissue May Link Macrophage Infiltration to Whole-Body Insulin Sensitivity

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J Clin Endocrinol Metab, published April 7, 2009, 10.1210/jc.2009-0002

ABSTRACT

Context: Adipose tissue in obesity is thought to be exposed to various stresses, predominantly in intraabdominal depots. We recently reported that p38MAPK and Jun N-terminal kinase (JNK), but not ERK and inhibitory- κ B kinase β , are more highly expressed and activated in human omental (OM) adipose tissue in obesity.

Objective: The aim was to investigate upstream components of the pathways that culminate in activation of p38MAPK and JNK.

Setting and Patients: Phosphorylation and expression of kinases were studied in paired samples of OM and sc adipose tissue from lean and obese subjects of two different cohorts ($n = 36$ and $n = 196$) by Western and real-time PCR analyses. The association with fat distribution, macrophage infiltration, insulin sensitivity, and glucose metabolism was assessed by correlation analyses.

Results: The amount of phosphorylated forms of the kinases provided evidence for an activated stress-sensing pathway consisting of the MAP3K Ask1 (but not MLK3 or Tak1), and the MAP2Ks MKK4, 3/6, (but not MKK7), specifically in OM. OM *Ask1*-mRNA was more highly expressed in predominantly intraabdominally obese persons and most strongly correlated with estimated visceral fat. Diabetes was associated with higher OM *Ask1*-mRNA only in the lean group. In OM, macrophage infiltration strongly correlated with *Ask1*-mRNA, but the obesity-associated increase in *Ask1*-mRNA could largely be attributed to the adipocyte cell fraction. Finally, multivariate regression analyses revealed OM-*Ask1* as an independent predictor of whole-body glucose uptake in euglycemic-hyperinsulinemic clamps.

Conclusions: An Ask1-MKK4-p38MAPK/JNK pathway may reflect adipocyte stress in response to adipose tissue inflammation, linking visceral adiposity to whole-body insulin resistance in obesity.

An In-Frame Deletion in Kir6.2 (KCNJ11) Causing Neonatal Diabetes Reveals a Site of Interaction between Kir6.2 and SUR1

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J Clin Endocrinol Metab, published April 7, 2009, 10.1210/jc.2009-0159

ABSTRACT

Context: Activating mutations in genes encoding the Kir6.2 (*KCNJ11*) and SUR1 (*ABCC8*) subunits of the pancreatic ATP-sensitive K^+ channel are a common cause of permanent neonatal diabetes (PNDM). All Kir6.2 mutations identified to date are missense mutations. We describe here a novel in-frame deletion (residues 28–32) in Kir6.2 in a heterozygous patient with PNDM without neurological problems that are detected by standard evaluation.

Objective: The aim of the study was to identify the mutation responsible for neonatal diabetes in this patient and characterize its functional effects.

Design: Wild-type and mutant Kir6.2/SUR1 channels were examined by heterologous expression in *Xenopus* oocytes.

Results: The Kir6.2–28 Δ 32 mutation produced a significant decrease in ATP inhibition and an increase in whole-cell K_{ATP} currents, explaining the diabetes of the patient. Tolbutamide block was only slightly reduced in the simulated heterozygous state, suggesting that the patient should respond to sulfonylurea therapy. The mutation decreased ATP inhibition indirectly, by increasing the intrinsic (unliganded) channel open probability. Neither effect was observed when Kir6.2 was expressed in the absence of SUR1, suggesting that the mutation impairs coupling between SUR1 and Kir6.2. Coimmunoprecipitation studies further revealed that the mutation disrupted a physical interaction between Kir6.2 and residues 1–288 (but not residues 1–196) of SUR1.

Conclusions: We report a novel *KCNJ11* mutation causing PNDM. Our results show that residues 28–32 in the N terminus of Kir6.2 interact both physically and functionally with SUR1 and suggest that residues 196–288 of SUR1 are important in this interaction.

The Tyrosine Kinase Receptor RET Interacts *in Vivo* with Aryl Hydrocarbon Receptor-Interacting Protein to Alter Survivin Availability

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J Clin Endocrinol Metab, published April 14, 2009, 10.1210/jc.2008-1980

ABSTRACT

Context: RET is a tyrosine kinase transmembrane receptor expressed in two main alternative isoforms: RET9 and RET51. RET transduces a positive signal leading to survival, differentiation, or migration in the presence of its ligand @FN Nonstandard abbreviations (such as GDNF) must be used more than twice to be retained in abstract. Text is treated separately. glial cell line-derived neurotrophic factor, whereas in its absence a proapoptotic fragment that initiates a negative signaling for apoptosis is generated. The signal transduction mechanisms leading to apoptosis are still unclear.

Objective: To shed light on the mechanisms of RET-induced apoptosis, we searched for novel interactors of RET51.

Design: The “split ubiquitin yeast two-hybrid system” was used with RET51 as bait against a human brain expression library.

Results: We identified aryl hydrocarbon receptor-interacting protein (AIP), a cochaperone recently found mutated in pituitary adenoma patients, as a novel interactor of RET. We showed that RET interacts specifically with AIP both in mammalian cell lines and *in vivo* in the pituitary gland, regardless of the presence of pituitary adenoma-specific mutations. *AIP* and *RET* genes were sequenced in 28 pituitary adenoma, but no relevant mutations were found. In addition, we identified the proapoptotic domain of RET as responsible for the interaction with AIP. Finally, we demonstrated that the AIP-RET interaction does not require RET kinase activity or kinase-dependent signal transduction and that it prevents the formation of the AIP-survivin complex.

Conclusions: The identification of the AIP-RET complex represents a starting point to study key cellular processes involved in RET-induced apoptosis.

Hypoglycemia from *IGF2* Overexpression Associated with Activation of Fetal Promoters and Loss of Imprinting in a Metastatic Hemangiopericytoma

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J Clin Endocrinol Metab, published April 21, 2009, 10.1210/jc.2009-0153

ABSTRACT

Context: The mechanism of *IGF2* overexpression in non-islet-cell tumor hypoglycemia is not understood.

Objective: We investigated the imprinting control and promoter usage for *IGF2* expression to identify a mechanism for increased IGF-II production in non-islet-cell tumor hypoglycemia.

Patient and Methods: A patient with metastatic hemangiopericytoma was studied. Tissue from the original hemangiopericytoma, metastatic tumor, and uninvolved liver was analyzed for IGF-II immunohistochemistry. *IGF2*, a paternally imprinted gene, shares a control region with maternally imprinted *H19*, a putative tumor suppressor. IGF-II and *H19* mRNA expression was compared in metastatic tumor and uninvolved liver by quantitative RT-PCR. Imprinting of *IGF2/H19* genes and *IGF2* promoter usage in metastatic tumor was investigated by RT-PCR and sequence analysis, and the methylation pattern in the *IGF2/H19* imprinting control region was analyzed.

Results: IGF-II protein expression was increased in metastatic tumor vs. uninvolved liver and original tumor. In the metastatic tumor, IGF-II mRNA was increased 60-fold, but *H19* mRNA was comparable to uninvolved liver; loss of imprinting of *IGF2*, but not *H19*, was identified; no major change in methylation of the *IGF2/H19* imprinting control regions was observed; and transcripts from four different *IGF2* promoters were detected, compared to two in uninvolved liver.

Conclusions: *IGF-2* overexpression, newly acquired in the metastatic tumor, was associated with loss of *IGF2* gene imprinting and different promoter usage. The imprinting control mechanism governing the *IGF2/H19* locus was intact, as evidenced by normal levels of *H19*, maintenance of *H19* imprinting, and no major change in methylation of the imprinting control regions.

The Paradoxical Increase in Cortisol Secretion Induced by Dexamethasone in Primary Pigmented Nodular Adrenocortical Disease Involves a Glucocorticoid Receptor-Mediated Effect of Dexamethasone on Protein Kinase A Catalytic Subunits

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(J Clin Endocrinol Metab, published April 21, 2009, 10.1210/jc.2009-0031)

ABSTRACT

Context: Primary pigmented nodular adrenocortical disease (PPNAD) results in most cases from mutations of the protein kinase A (PKA) regulatory subunit 1A (*PRKAR1A*) gene. Patients with PPNAD exhibit a paradoxical increase in cortisol secretion in response to dexamethasone.

Objective: The aim was to investigate the mechanism of the action of dexamethasone on adrenocortical cells removed from patients with PPNAD and a transgenic model of PPNAD [Tg(tTA/X2AS) mice].

Design and Setting: We performed an *in vitro* study in an academic research laboratory.

Patients: Eleven patients with histologically proven PPNAD were included in the study.

Intervention: Cultured PPNAD cells were incubated with dexamethasone in the presence of various modulators of the cAMP/PKA pathway and the glucocorticoid receptor antagonist RU486.

Main Outcome Measure: Cortisol and corticosterone were measured by radioimmunological assays in cell culture supernatants.

Results: Dexamethasone stimulated *in vitro* cortisol secretion from PPNAD tissues in six patients. The stimulatory effect of dexamethasone on cortisol release was not reduced by the adenylyl cyclase inhibitor SQ22536 or potentiated by the phosphodiesterase inhibitor IMBX and the cAMP analog 8Br-cAMP. Conversely, the PKA inhibitor H89 and RU486 inhibited the cortisol response to dexamethasone. Dexamethasone had no effect on cortisol production from normal human adrenocortical cells but stimulated corticosteroidogenesis in the presence of RU486. Similarly, dexamethasone failed to influence corticosterone release by adrenocortical cells removed from Tg(tTA/X2AS) mice but stimulated corticosteroidogenesis in the presence of RU 486.

Conclusions: These results indicate that, in human PPNAD tissues, dexamethasone paradoxically stimulates cortisol release through a glucocorticoid receptor-mediated effect on PKA catalytic subunits.

MBX-102/JNJ39659100, a Novel Peroxisome Proliferator Activated Receptor- γ Ligand with Weak Transactivation Activity Retains Full Anti-Diabetic Properties in the Absence of Side Effects

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(Mol Endocrinol, published April 23, 2009, 10.1210/me.2008-0473)

ABSTRACT

MBX-102/JNJ39659100 (MBX-102) is in clinical development as an oral glucose lowering agent for the treatment of type 2 diabetes. MBX-102 is a non-thiazolidinedione (TZD) selective partial agonist of PPAR- γ that is differentiated from the TZDs structurally, mechanistically, pre-clinically and clinically. In diabetic rodent models, MBX-102 has insulin sensitizing and glucose lowering properties comparable to TZDs without dose-dependent increases in body weight. *In vitro*, in contrast with full PPAR- γ agonist treatment, MBX-102 fails to drive human and murine adipocyte differentiation and selectively modulates the expression of a subset of PPAR- γ target genes in mature adipocytes. Moreover, MBX-102 does not inhibit osteoblastogenesis of murine mesenchymal cells. Compared to full PPAR- γ agonists, MBX-102 displays differential interactions with the PPAR- γ ligand binding domain (LBD) and possesses reduced ability to recruit coactivators. Interestingly, in primary mouse macrophages, MBX-102 displays enhanced anti-inflammatory properties compared to other PPAR- γ or α/γ agonists suggesting that MBX-102 has more potent transrepression activity. In summary, MBX-102 is a selective PPAR- γ modulator with weak transactivation but robust transrepression activity. MBX-102 exhibits full therapeutic activity without the classical PPAR- γ side effects and may represent the next generation insulin sensitizer.

Fasting-Induced Hepatic Production of DHEA is Regulated by PGC-1 α , ERR α and HNF4 α

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(*Mol Endocrinol*, published April 23, 2009, 10.1210/me.2009-0024)

ABSTRACT

The transcriptional coactivator PGC-1 α is involved in the coordinate induction of changes in gene expression in the liver that enable a homeostatic response to alterations in metabolic state, environmental cues and nutrient availability. In exploring the specific pathways under PGC-1 α regulation in the liver, we have made the surprising observation that this coactivator can induce the expression of CYP11A1 and CYP17A1, key rate limiting enzymes involved in the initial steps of steroidogenesis. Both of these enzymes function to produce C19-steroids, converting cholesterol into pregnenolone, and then to DHEA. ERR α mediates PGC-1 α 's induction of CYP11A1 and binds within the first intron of the CYP11A1 gene. Both ERR α and HNF4 α are required for PGC-1 α -mediated induction of CYP17A1 and specific binding sites for these receptors have been identified in the regulatory regions of this gene. The potential physiological significance of these observations was highlighted in rats where fasting induced hepatic expression of PGC-1 α and CYP17A1 and was associated with an increase in hepatic levels of DHEA. These data suggest that DHEA could be playing a role as an intracellular signaling molecule involved in modulating hepatic activity in response to fasting conditions.

Characterization of ASC-2 as an Anti-Atherogenic Transcriptional Coactivator of Liver X Receptors in Macrophages

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(*Mol Endocrinol*, published April 2, 2009, 10.1210/me.2008-0308)

ABSTRACT

Activating signal cointegrator-2 (ASC-2) functions as a transcriptional coactivator of many nuclear receptors and also plays important roles in the physiology of the liver and pancreas by interacting with liver X receptors (LXRs), which antagonize the development of atherosclerosis. This study was undertaken to establish the specific function of ASC-2 in macrophages and atherogenesis. Intriguingly, ASC-2 was more highly expressed in macrophages than in the liver and pancreas. To inhibit LXR-specific activity of ASC-2, we used DN2, which contains the C-terminal LXXLL motif of ASC-2 and thereby acts as an LXR-specific, dominant-negative mutant of ASC-2. In DN2-overexpressing transgenic (Tg) macrophages, cellular cholesterol content was higher and cholesterol efflux lower than in control macrophages. DN2 reduced LXR ligand-dependent increases in the levels of ABCA1, ABCG1, and apoE transcripts, as well as the activity of luciferase reporters driven by the LXR response elements (LXREs) of ABCA1, ABCG1, and apoE genes. These inhibitory effects of DN2 were reversed by overexpression of ASC-2. Chromatin immunoprecipitation analysis demonstrated that ASC-2 was recruited to the LXREs of the ABCA1, ABCG1, and apoE genes in a ligand-dependent manner and that DN2 interfered with the recruitment of ASC-2 to these LXREs. Furthermore, low density lipoprotein receptor (LDLR)-null mice receiving bone marrow transplantation from DN2-Tg mice showed accelerated atherogenesis when administered a high-fat diet. Taken together, these results indicate that suppression of the LXR-specific activity of ASC-2 results in both defective cholesterol metabolism in macrophages and accelerated atherogenesis, suggesting that ASC-2 is an anti-atherogenic coactivator of LXRs in macrophages.

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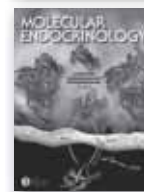
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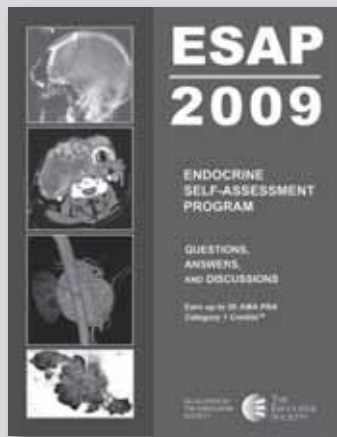
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3. A curriculum vitae for each author, including a full bibliography of published work
4. A completion date for the manuscript.

These items should be e-mailed to endoreviews@endo-society.org. Do not upload them to Rapid Review.

Once a proposal has been reviewed by the editors, the author will be notified of its status.

Manuscript Submission Procedures

Please submit your manuscript to *Endocrine Reviews* through the Rapid Review system at the following site: <https://www.rapidreview.com/tes/CALogon.jsp>. If this is your first submission through E-Review, click on "New to Rapid Review?" to create an author account. If you already have an account from a previous submission, enter your username and password to submit a new or revised manuscript. If you have forgotten your username and/or password, e-mail the editorial office (endoreviews@endo-society.org) for assistance. Note that your author account is the same for *JCEM*, *Endocrinology*, *Molecular Endocrinology*, and *Endocrine Reviews*. Authors should be aware that in submitting a manuscript for consideration by *Endocrine Reviews*, they are submitting their paper to The Endocrine Society Central Journals Office database, which is accessible by the Editors-in-Chief of all the Society's journals.

All submissions must include:

- Cover letter stating the authors' wish that the manuscript be evaluated for publication in *Endocrine Reviews*. This letter must list the title, names of all authors of the paper, and the submission package contents and suggest specific reviewers. Elsewhere on the submission form authors may suggest up to five (5) specific reviewers and/or request the exclusion of up to three (3) others.
- Completed Copyright Assignment & Affirmation of Originality form. This form should be faxed to the Editorial Office at 301-951-2617 and should include the manuscript number in the space provided on the form.
- Completed Disclosure of Potential Conflict of Interest form. The corresponding author must acquire all of the authors' completed disclosure forms and fax them, together, to the editorial office along with the Author Disclosure Summary. Revised manuscripts will not be processed until all signatures and the summary are received.

All submissions must include:

- Cover letter stating the authors' wish that the manuscript be evaluated for publication in *Endocrine Reviews*. This letter must list the title, names of all authors of the paper, and the submission package contents and suggest specific reviewers. Elsewhere on the submission form authors may suggest up to five (5) specific reviewers and/or request the exclusion of up to three (3) others.
- Completed Copyright Assignment & Affirmation of Originality form. This form should be faxed to the Editorial Office at 301-951-2617 and should include the manuscript number in the space provided on the form.
- Completed Disclosure of Potential Conflict of Interest form. The corresponding author must acquire all of the authors' completed disclosure forms and fax them, together, to the editorial office along with the Author Disclosure Summary. Revised manuscripts will not be processed until all signatures and the summary are received.

If you need any assistance with the submission of your paper, please contact the Editorial Office at 301-951-2603, or endoreviews@endo-society.org.

Manuscript Preparation

General Format

The Journal requires that all submissions be submitted in a two-column format that follows these guidelines:

- Format text from the Introduction through the Acknowledgements in two single-spaced columns. All text should be single spaced with one-inch margins on both sides using 11-point type in Times Roman font.
- The abstract, references, and legends should be set in one column.
- All tables and figures must be placed after the text and must be labeled. Submitted papers must be complete, including the title page, abstract, figures, and tables. Papers submitted without all of these components will be placed on hold until the manuscript is complete.

Title Page

The title page should include the following:

- Full title (a concise statement of the article's major contents)
- Authors' names and institutions. At least one person must be listed as an author; no group authorship without a responsible party is allowed. A group can be listed in the authorship line, but only on behalf of a person or persons. All group members not listed in the authorship line must be listed in the acknowledgment.
- All papers with U.S. National Institutes of Health funding must include the paragraph indicated in the *The Endocrine Society NIH statement*.
- Abbreviated title of not more than 40 characters for page headings
- At least three key terms for indexing and information retrieval
- Word count (excluding abstract, figure captions, and references)
- Corresponding author's e-mail and ground mail addresses, telephone and fax numbers

- Name and address of person to whom reprint requests should be addressed
- Any grants or fellowships supporting the writing of the paper

Abstract

Please use the following guidelines when preparing the abstract:

- Do not exceed 250 words.
- Briefly describe in complete sentences the purpose of the investigation, the methods used, the results obtained, and the principal conclusions.
- Do not refer to the text or references.
- Write the abstract with a general audience in mind.

Outline

An outline of the manuscript's contents helps to lead the reader by providing preliminary information on the structure of the review.

Introduction

The article should begin with a brief introductory statement that places the work to follow in historical perspective and explains its intent and significance.

Body of Manuscript

The organization of this privileged section of the review is left to the authors. However, the organization should be logical and readily comprehended by the reader. As indicated above, an outline that indicates this organization is desirable.

Acknowledgments

The acknowledgment section should include the names of those people who contributed to a study but did not meet the requirements for authorship. The corresponding author is responsible for informing each person listed in the Acknowledgments section that they have been included and providing them with a description of their contribution so that they know the activity for which they are considered responsible.

References

References to the literature should be cited in numerical order (in parentheses) in the text and listed in the same numerical order at the end of the manuscript on a separate sheet or sheets. There must be only one reference to a number.

The number of references cited should be kept to a reasonable minimum; to this end, appropriate recent reviews should be cited whenever possible.

Examples of the reference style that should be used are given below. The titles of journals should be abbreviated according to the style used in the *Index Medicus*.

Journal articles and abstracts: List all authors. The citation of unpublished observations, of personal communications, and of manuscripts in preparation or submitted for publication is not permitted in the bibliography. Such citations should be inserted at appropriate places in the text, in parentheses and without serial number, or be presented in the footnotes. The citation of manuscripts in press (*i.e.*, accepted for publication) is permitted in the bibliography; the name of the journal in which they appear must be supplied. If references to personal communications are made, authors are encouraged to keep written proof of the exchange. If it is necessary to cite an abstract because it contains substantive data not published elsewhere, it must be designated at the end of the reference [e.g., 68:313 (Abstract)]. **The author is responsible for the accuracy of references.**

Books: List all authors or editors.

Sample References

1. Binoux M, Hossenlopp P 1986 Insulin-like growth factor (IGF) and IGF-binding proteins: comparison of human serum and lymph. *J Clin Endocrinol Metab* 67:509–514

2. MacLaughlin DT, Cigarros F, Donahoe PK 1988 Mechanism of action of Mullerian inhibiting substance. Program of the 70th Annual Meeting of The Endocrine Society, New Orleans, LA, 1988, p 19 (Abstract P1-21)
3. Bonneville F, Cattin F, Diemann J-L 1986 Computed tomography of the pituitary gland. Heidelberg: Springer-Verlag; 15–16
4. Burrow GN 1987 The Thyroid: nodules and neoplasia. In: Felig P, Baxter JD, Broadus AE, Frohman LA, eds. *Endocrinology and metabolism*. 2nd ed. New York: McGraw-Hill; 473–507

For general aid in the preparation of manuscripts, authors should consult: CBE Style Manual: A Guide for Authors, Editors and Publishers. 5th ed. Bethesda, MD: Council of Biology Editors; 1983

Tables

Tables must be constructed as simply as possible and be intelligible without reference to the text. Each table must have a concise heading. A description of experimental conditions may appear together with footnotes at the foot of the table. Tables must not simply duplicate the text or figures. The width of the table must be designed to occupy one or two journal columns, with no more than four (4) table columns or eight (8) to 10 table columns, respectively.

Figures and Legends

Please review the detailed instructions for preparing digital art at <http://art.cadmus.com/da/index.jsp>. E-mail queries can be sent to digitalart@cadmus.com. All figures must display the figure number.

Sizing the figure: The author is responsible for providing digital art that has been properly sized, cropped, and has adequate space between images. Plan the size of the figure to fill 1, 1.5, or 2 columns in the printed journal (see chart below for dimensions). In most cases, figures should be prepared for 1-column width. Produce original art at the size it should appear in the printed journal. (Note for PowerPoint users: The sizing instructions do not apply if you are submitting PowerPoint files for print production in E-Review. On the submission page, check boxes to indicate that the figures are the correct size and resolution.)

1 column = 18 picas, 7.5 cm, 3.0 in

1.5 columns = 30 picas, 12.5 cm, 5.0 in

2 columns = 38 picas, 16.0 cm, 6.5 in

Lettering: At 100% size, no lettering should be smaller than 8 point (0.3 cm high) or larger than 12 point (0.4 cm high). Use bold and solid lettering. Lines should be thick, solid, and no less than 1-point rule. Avoid the use of reverse type (white lettering on a darker background). Avoid lettering on top of shaded or textured areas. Titles should be clear and informative. Keep wording on figures to a minimum, and confine any explanation of figures to their separate-page legends. Label only one vertical and one horizontal side of a figure. *Freehand lettering or drawing is unacceptable.*

Color figures: Figures should now be submitted as RGB (red, green, blue) format. Saving color figures to this format will be more convenient for authors as RGB is the standard default on most programs. Color images will be preserved as RGB up until the time of printing and will be posted online in their original RGB form. Using RGB color mode for online images will be a significant improvement for figures that contain fluorescent blues, reds, and greens. Therefore the online journal will accurately reflect the true color of the images the way the author intended. For print, the images will be converted to CMYK through an automated color conversion process.

Shading: Avoid the use of shading, but if unavoidable, use a coarse rather than a fine screen setting (80–100 line screen is preferred). Avoid 1–20% and 70–99% shading; make differing shades vary by at least 20%, *i.e.*, 25%, 45%, 65%. Instead of shading, denote variations in graphs or drawings by cross-hatching; solid black; or vertical, horizontal, or diagonal striping. Avoid the use of dots.

Grouped figures: For grouped figures, indicate the layout in a diagram. Place grouped figures so that they can be printed in 1 column width with uniform margins. Indicate magnification in the legends and by internal reference markers in the photographs. Their length should

represent the fraction or multiple of a micrometer, appropriate to the magnification.

Graphs: Graphs with axis measures containing very large or small numbers should convert to easily readable notations. *Example:* For an ordinate range of “counts per minute” values from 1,000 to 20,000, the true value may be multiplied by 10^{-3} (scale would read from 1 to 20) and the ordinate axis display “cpm ($\times 10^{-3}$).” Similarly, for a Scatchard plot with values ranging from 0.1 to 2 femtomolar (10^{-15} M), the scale may run from 0.1 to 2 with the abscissa labeled “m($\times 10^{-15}$).” *Three-dimensional bar graphs will not be published if the information they refer to is only two-dimensional.*

Supplemental Data

Supplemental Data allows authors to enhance papers in *Endocrine Reviews* by making additional substantive material available to readers. Supplemental Data may take the form of figures, tables, datasets, derivations, or videos and is published only in *Endocrine Reviews* online; it does not appear in the printed version of the journal. Authors who wish to include Supplemental Data should state so in the cover letter when the manuscript is submitted.

Supplemental Data files should be submitted through Rapid Review at the time of manuscript submission and will be reviewed along with the manuscript. The files should be uploaded in the field marked “Upload Supplemental Data Files”, and should NOT be attached with the manuscript and figure files. Authors should refer to the Supplemental Data in the manuscript at an appropriate point in the text or figure/table legend.

The file formats listed below may be used for Supplemental Data. Provide a brief description of each item in a separate HTML or Word file (*i.e.*, figure or table legends, captions for movie or sound clips, etc.). Do not save figure numbers, legends, or author names as part of an image. File sizes should not exceed 5 MB. Images should not exceed 500 pixels in width or height. Do not use tabs or spaces for Word or WordPerfect tables; please use the table functions available within these word processing programs to prepare tables. For web pages, provide a complete list of files and instructions for creating directories.

.htm, HTML*
.jpg, JPEG image*
.gif, Graphical image
.pdf, Adobe Portable Document Format
.xls, MS Excel Spreadsheet
.mov, Quick Time
.wav, Sound
.doc, MS Word 6 documents**
.txt, Plain ASCII*

*These files can be viewed directly on standard web browsers.

**MS Word may be used for text only.

Units of Measure

All nonstandard abbreviations in the text must be defined immediately after the first use of the abbreviation. The list of *Standard Abbreviations* is given in the link.

Editorial Policies and Guidelines

Prior Publication

Failure to notify the editor that some results in the manuscript are being or have been previously published will result in placement of a notice in the journal that the authors have violated the Ethical Guidelines for Publication of Research in The Endocrine Society Journals. The journal publishes original research and review material. Material previously published in whole or in part shall not be considered for publication. At the time of submission, authors must divulge in their cover letter all prior publications or postings of the material in any form of media. Abstracts or posters displayed for colleagues at scientific meetings need not be reported. Other postings of any part of the submitted material on web pages, as well as those essential for participation in required registries will be evaluated by the Editor-In-Chief, who shall

determine if those postings are material enough to constitute prior publication.

Authorship Criteria

An author should have participated in either the conception or planning of the work, the interpretation of the results and the writing of the paper. An acknowledgment accompanying the paper is appropriate recognition for others who have contributed to a lesser extent, *e.g.*, provision of clones, antisera or cell lines, or reading and reviewing manuscripts in draft. The signature of each author on the Affirmation of Originality and Copyright Release form that must be submitted with the manuscript indicates that all authors have had a part in the writing and final editing of the report, all have been given a copy of the manuscript, all have approved the final version of the manuscript, and all are prepared to take public responsibility for the work, sharing responsibility and accountability for the results.

Guidelines for Considering Authors of Non-research Articles who have a Potential COI

The editors of The Endocrine Society’s journals appreciate the importance of assuring unbiased authorship of editorials, reviews, and other non-research features involving selection of evidence to be discussed and perspectives to be presented. Consequently, special care is taken in choosing authors for such articles to assure their views are balanced and unencumbered, and that the Society’s policies on disclosure of conflicts of interest are implemented.

Obligations of Reviewers

The critical and confidential review of manuscripts is an essential element of research publications. Every scientist has an obligation to contribute to the peer review process by serving as a reviewer. Among the obligations of reviewers is the commitment to providing an expert, critical, and constructive scientific and literary appraisal of research reports in their fields of knowledge, skills, and experience in a fair and unbiased manner. In order to facilitate the prompt sharing of scientific results, it is also the obligation of each reviewer to complete their assignments promptly, within the editor’s deadline. Should a delay in their review occur, the reviewer has the obligation to notify the editor at once.

Reviewers should not review a manuscript if: 1) they do not think that they are competent to assess the research described, 2) they believe there is a conflict of interest or personal or professional relationship with the author(s) that might bias their assessment of the manuscript, or (3) there is any other situation that could bias their review. Employment at the same institution as one of the authors does not automatically represent a conflict. Having previously reviewed the article for another journal does not disqualify a reviewer, although the editor should be informed so the reviewer’s perspective can be considered. In circumstances when reviewers need to recuse themselves, they should notify the editor promptly, preferably with an explanation. If reviewers are uncertain whether they should recuse themselves, they should consult with the editor.

The reviewer should strive to provide accurate, detailed, and constructive criticisms, and the review should be supported by appropriate references, especially if unfavorable. The reviewer should also note whether the work of others is properly cited. If the reviewer notes any substantial resemblance of the manuscript being reviewed to a published paper or to a manuscript submitted at the same time to another journal, they should promptly report this to the editor.

No part of the manuscript under review should ordinarily be revealed to another individual without the permission of the editor. If a reviewer consults a colleague on a particular point, this fact, and the name of the collaborator or consultant, should be reported to the editor, preferably in advance. With these exceptions, a reviewer must obtain through the editor written permission from the authors to use or disclose any of the unpublished content of a manuscript under review.

Standard Abbreviations

(Note that periods are omitted in most abbreviations. Units of measure should not be abbreviated if they do not appear with a specific amount. Abbreviations for elements listed on the periodic table of elements are considered standard abbreviations.)

acquired immunodeficiency syndrome	AIDS	growth hormone (somatotropin)	GH	phosphate-buffered saline	PBS
adenosine 5'-mono-, di-, and triphosphates	AMP, ADP, ATP	GH-releasing hormone	GHRH	polyacrylamide gel electrophoresis	PAGE
adrenocorticotropin	ACTH	guanosine 5'-mono-, di-, and triphosphates	GDP, GMP, GTP	polymerase chain reaction	PCR
analysis of variance	ANOVA	GTP-binding protein	G protein	probability	P
bovine serum albumin	BSA	<i>N</i> -2-hydroxyethylpiperazine- <i>N'</i> -2-ethane sulfonic acid	HEPES	PTH-related peptide	PTHrP
complementary DNA	cDNA	high-performance liquid chromatography	HPLC	radioimmunoassay	RIA
complementary RNA	cRNA	human immunodeficiency virus	HIV	reverse transcription-polymerase chain reaction	RT-PCR
corticotropin-releasing hormone	CRH	5-hydroxy-indole-acetic acid	5-HIAA	ribonucleic acid	RNA
3',5'-cyclic AMP	cAMP	immunoglobulin	Ig	ribosomal RNA	rRNA
cytidine 5'-mono-, di-, and triphosphates	CMP, CDP, CTP	insulin-like growth factor	IGF	sex hormone-binding globulin	SHBG
deoxyribonucleic acid	DNA	interleukin	IL (<i>e.g.</i> , IL-1, IL-6)	sodium dodecyl sulfate-polyacrylamide gel electrophoresis	SDS-PAGE
Dulbecco's modified Eagle's medium	DMEM	intramuscular(-ly)	im	standard deviation	SD
electrophoretic mobility shift assay	EMSA	intraperitoneal(-ly)	ip	standard error	SE
enzyme-linked immunosorbent assay	ELISA	intravenous(-ly)	iv	standard error of the mean	SEM
ethylenediamine tetra-acetate	EDTA	luteinizing hormone	LH	subcutaneous(-ly)	sc
ethyleneglycol-bis-(β -aminoethyl ether)- <i>N,N,N',N'</i> -tetraacetic acid	EGTA	log of the odds	LOD	thyrotropin	TSH
extracellularly regulated kinase	ERK	median effective concentration	EC ₅₀	thyroxine	T ₄
follicle-stimulating hormone	FSH	median effective dose	ED ₅₀	3,5,3'-triiodothyronine	T ₃
gonadotropin-releasing hormone	GnRH (LHRH)	median inhibitory concentration	IC ₅₀	3,3',5'-triiodothyronine	rT ₃
		median lethal dose	LD ₅₀	transfer RNA	tRNA
		melanocyte-stimulating hormone	MSH	transforming growth factor	TGF
		messenger RNA	mRNA	tris(hydroxymethyl)-aminomethane	Tris
		minimal essential medium	MEM	TSH-releasing hormone	TRH
		mitogen-activated protein kinase	MAPK	tumor necrosis factor	TNF
		optical density	OD	ultraviolet	UV
		parathyroid hormone	PTH	versus	<i>vs.</i>

Nomenclature of Steroids

1. *Steroids* should be named, wherever possible, according to the IUPAC-IUB 1971 Definitive Rules for Steroid Nomenclature (*Pure Appl Chem* 31:285–322, 1972).

2. Trivial names may be used but, with the exception of cholesterol, estrone, 17 β -estradiol, estriol, aldosterone, androsterone, etiocholanolone, dehydroepiandrosterone, 5 α -dihydrotestosterone, testosterone, androstenedione, pregnenolone, progesterone, corticosterone, deoxycorticosterone, cortisone, cortisol, must be defined systematically in a

single footnote. This footnote should also contain the definitions of all letter abbreviations.

3. Trivial names may be modified by prefixes indicating substituents (as in 17-hydroxyprogesterone for 17-hydroxy-4-pregnene-3,20-dione), double bonds (as in 7-dehydrocholesterol for 5,7-cholestadien-3 β -ol) and epimeric configurations of functional groups provided the locus of epimerization is indicated (as in 3-epiandrosterone for 3 β -hydroxy-5 α -androstane-17-one).

Nomenclature of Vitamin D Metabolites: Analogous and Structurally Related Compounds

vitamin D₃, cholecalciferol
 vitamin D₂, ergocalciferol
 25-hydroxyvitamin D₃, 25-hydroxycholecalciferol, 25OHD₃
 25-hydroxyvitamin D₂, 25-hydroxyergocalciferol, 25OHD₂
 25-hydroxyvitamin D, D₃, D₂ unspecified, 25-OHD
 1,25-dihydroxyvitamin D₃, 1,25-dihydroxycholecalciferol, 1,25-(OH)₂D₃
 1,24,25-trihydroxyvitamin D₃, 1,24,25-trihydroxycholecalciferol, 1,24,25-(OH)₃D₃
 24,25-dihydroxyvitamin D₃, 24,25-dihydroxycholecalciferol, 24,25-(OH)₂D₃

25,26-dihydroxyvitamin D₃, 25,26-dihydroxycholecalciferol, 25,26-(OH)₂D₃
 1,25-dihydroxyvitamin D₂, 1,25-dihydroxyergocalciferol, 1,25-(OH)₂D₂
 1,25-hydroxyvitamin D, D₃, D₂ unspecified, 1,25-OHD
 dihydrotachysterol₃, DHT₃
 dihydrotachysterol₂, DHT₂
 25-hydroxydihydrotachysterol₃, 25-OH-DHT₃
 25-hydroxydihydrotachysterol₂, 25-OH-DHT₂

Digital Image Integrity

When preparing digital images, authors must adhere to the following guidelines as stated in The CSE's White Paper on Promoting Integrity in Scientific Journal Publications:

- No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.
- Adjustments of brightness, contrast, or color balance are acceptable if

they are applied to the entire image and as long as they do not obscure, eliminate, or misrepresent any information present in the original.

- The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (e.g., dividing lines) and in the figure legend.

Deviations from these guidelines will be considered as potential ethical violations.

Note that this is an evolving issue, but these basic principles apply regardless of changes in the technical environment. Authors should be aware that they must provide original images when requested to do so by the Editor-in-Chief who may wish to clarify an uncertainty or concern.

[Please see paper of Rossner and Yamada (Journal of Cell Biology, 2004, 166:11–15), which was consulted in developing these policy issues, for additional discussion and The CSE's White Paper on Promoting Integrity in Scientific Journal Publications, published by The Council of Science Editors, 2006.]

Publication and Production Guidelines

Proofs and Reprints

Proofs and a reprint order form are sent to the corresponding author unless the Editorial Office is advised otherwise. The author should designate by footnote on the title page of the manuscript the name and address of the person to whom reprint requests should be directed.

Questions about reprints should be referred to Cadmus Professional Communications at 410-819-3912 (direct) or 800-407-9190 (toll-free).

Page and Color Charges

There is no submission fee for The Endocrine Society journals.

There will be a charge of \$235 per color figure for members of The Endocrine Society and \$735 per color figure for non-members.

Authors must submit usable digital art that passes Cadmus's Rapid Inspector. Queries on page charges may be directed to Joy Carter at Cadmus Professional Communications (410-691-6439; fax 410-684-2792).

NIH Deposits and Institutional Repositories

For articles that were funded by NIH, accepted manuscripts will be submitted to PubMed Central. These manuscripts will be made freely available online twelve months after print publication. NIH will contact the author to confirm submission.

Ethical Guidelines for Research¹

Expectation of Ethical Conduct

The Endocrine Society's mission is to advance excellence in endocrinology and be an integrative force in scientific research and medical practice. Such progress depends on integrity in the conduct of scientific research and truthful representation of findings. Specific guidelines regarding the Society's expectations for ethical conduct can be found in the Code of Ethics of The Endocrine Society and the Ethical Guidelines for Publications of Research.

The journal editors and publication oversight committees of The Endocrine Society are dedicated to upholding high ethical standards in its publications and expect authors and reviewers to do the same.

Editorial Prerogative for Ethical Unsuitability

The editors reserve the right to reject manuscripts describing research that does not meet acceptable standards of research behavior as determined by the Belmont Report, the Geneva Convention, the Declaration of Helsinki, and The Endocrine Society Code of Ethics.

Experimental Subjects

All studies involving human subjects or human tissue must be in accordance with the principles set out in the Declaration of Helsinki and must have been formally approved by the appropriate institutional review board, ethical review committee, or equivalent. All manuscripts should indicate that such approval was obtained. The study populations should be described in detail. In many studies details of age, race, and sex are important. In experiments involving any significant risk or discomfort to subjects, it should be documented that informed consent was obtained from the subjects and that an institutional human research committee had approved the investigations. In text, tables and figures subjects must be identified by number or letter rather than by initials or names. Photographs of patients' faces should be included only if scientifically relevant. Authors should obtain written consent from the patient for use of such photographs.

Guidelines for the Care and Use of Experimental Animals

The Society requires that all studies involving the use of animals published in its journals be conducted in accordance with mandated standards of humane care. The appropriateness of the experimental procedures, as well as the species and required number of animals used, must be considered in the design of any study. All research animals must be acquired and used in compliance with federal, state, and local laws and institutional regulations. In particular, the Society recommends that animals be maintained in accordance with the NIH Guide for the Care and Use of Laboratory Animals [1996 (7th ed.) Washington, DC:

National Academy Press, aka National Research Council Guide.]

Research animals must receive appropriate tranquilizers, analgesics, anesthetics and care to minimize pain and discomfort during preoperative, operative, and postoperative procedures. The choice and use of drugs must be made in accordance with the NIH Guide. Where the use of anesthetics would negate the results of the experiment, the protocol must be clearly justified and approved by the Committee on Animal Care and Use of the local institution and according to accepted veterinary medical practice. The health of the animals must be properly monitored. If either the study or the condition of the animals requires that they be killed, it shall be done in a humane manner.

The manuscript must indicate that the studies were approved by the authors' institutional committee on animal care.

Ethical Guidelines for Publication of Research in The Endocrine Society Journals

The Publications Committee is keenly aware of the importance of formulating and disseminating rules of good conduct for authors, reviewers, and editors. Equally important is the establishment of due process for alleged or apparent improprieties. The Council of The Endocrine Society has approved the following Ethical Guidelines as prepared by the Publications Committee and has authorized periodic publication as well as distribution to members of our Editorial Boards, reviewers, and authors submitting manuscripts. The following statement is not meant to be all-inclusive but is provided in sufficient detail to give a clear understanding of ethical considerations to all concerned.

Introduction

The fundamentals of good conduct as they apply to research are honesty, fairness, good manners, and the subordination of self-interest to the common interest of our profession and our society. In these notes, the Publications Committee sets forth its rules of good conduct for authors, reviewers, and editors.

Obligations of Authors

Authorship Conditions

An author should have participated in either the conception or planning of the work, the interpretation of the results and the writing of the paper. An acknowledgment accompanying the paper is appropriate recognition for others who have contributed to a lesser extent, e.g., provision of clones, antisera or cell lines, or reading and reviewing manuscripts in draft. The signature of each author on the Affirmation of Originality and Copyright Release form that must be submitted with the manuscript indicates that all authors have had a part in the writing and final editing of the report, all have been given a copy of the manuscript, all have approved the final version of the manu-

¹ Approved by Council October 2007; posted January 2008.

script, and all are prepared to take public responsibility for the work, sharing responsibility and accountability for the results.

Authorship Obligations

The foremost obligation of an author is to present a clear, honest, accurate, and complete account of the research performed. Each manuscript should describe a complete study or a completed phase of an extended study. Fragmentation of reports should be avoided. When some of the results are to appear in another journal, in publications of congresses, symposia, workshops, etc., details plus a copy of the other paper(s) should be supplied to the editor. Any preliminary accounts or abstracts of the work, already published, must be referenced in the complete report.

The author has an obligation to: 1) describe the work in sufficient detail to allow others to repeat the work; 2) adhere to the journals' policy regarding preparation of digital images as outlined below; 3) include all relevant data, including those which may not support the hypothesis being tested; 4) cite those publications which have a direct bearing on the novelty and interpretation of the results; 5) make unique resources available to other investigators for academic research purposes, as a condition of publication. The Endocrine Society endorses the philosophy of open exchange of research materials and requires this; 6) ensure no substitution, addition, or deletion of data or text during the proof correction process (after acceptance). Answers to author queries and changes to typographical or printer's errors may be made to proofs. Any other changes will require that the proofs be returned to the editorial office for re-review of the manuscript; 7) If there are any additions, deletions, or changes in position of the names that appear in the authorship line of the originally submitted manuscript, the corresponding author must send to the Editorial Office a brief letter, signed by all authors, stating that they agree to the change.

Image Integrity

When preparing digital images, authors must adhere to the following guidelines (as stated in Reference 8):

- No specific feature within an image may be enhanced, obscured, moved, removed, or introduced.
- Adjustments of brightness, contrast, or color balance are acceptable if they are applied to the entire image and as long as they do not obscure, eliminate, or misrepresent any information present in the original.
- The grouping of images from different parts of the same gel, or from different gels, fields, or exposures must be made explicit by the arrangement of the figure (e.g., dividing lines) and in the figure legend.

Deviations from these guidelines will be considered as potential ethical violations.

Note that this is an evolving issue, but these basic principles apply regardless of changes in the technical environment. Authors should be aware that they must provide original images when requested to do so by Editors-in-Chief who may wish to clarify an uncertainty or concern.

Please see paper of Rossner and Yamada (*Journal of Cell Biology*, 2004, 166:11–15), which was consulted in developing these policy issues, for additional discussion.

Scientific Misconduct and Unethical Practices

The Endocrine Society accepts the definitions of plagiarism, fabrication and falsification proposed in the *ORI Handbook for Institutional Research Integrity Officers*, February 1997, Office of Research Integrity, Public Health Service of the U.S. Department of Health and Human Services (Washington, DC). Scientific misconduct and unethical acts include, but are not limited to, plagiarism, fabrication, falsification, redundant or duplicate publication, violation of federal, state or institutional rules, and honorary authorship.

The list below includes, but is not limited to, the following prohibited acts:

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The Endocrine Society Publications Committee will investigate any breach of these policies and determine appropriate action.

Obligations of Reviewers

The critical and confidential review of manuscripts is an essential element of research publications. Every scientist has an obligation to contribute to the peer review process by serving as a reviewer. Among the obligations of reviewers is the commitment to providing an expert, critical, and constructive scientific and literary appraisal of research reports in their fields of knowledge, skills, and experience in a fair and unbiased manner. In order to facilitate the prompt sharing of scientific results, it is also the obligation of each reviewer to complete their assignments promptly, within the editor's deadline. Should a delay in their review occur, the reviewer has the obligation to notify the editor at once.

Reviewers should not review a manuscript if: 1) they do not think that they are competent to assess the research described, 2) they believe there is a conflict of interest or personal or professional relationship with the author(s) that might bias their assessment of the manuscript, or (3) there is any other situation that could bias their review. Employment at the same institution as one of the authors does not automatically represent a conflict. Having previously reviewed the article for another journal does not disqualify a reviewer, although the editor should be informed so the reviewer's perspective can be considered. In circumstances when reviewers need to recuse themselves, they should notify the editor promptly, preferably with an explanation. If reviewers are uncertain whether they should recuse themselves, they should consult with the editor.

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The Editor-in-Chief directs and supervises the policies of a journal and is responsible for maintaining its scientific and literary quality. The first obligation of an Editor-in-Chief and the journal Editors is to make certain that all authors receive confidential, expert, critical, and unbiased reviews of their work in a timely fashion. The editors and members of the editor's staff should not disclose any information about a manuscript submitted for review to anyone except the reviewers or authors or those working on their behalf.

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It is now recognized that manuscripts appearing in Rapid Electronic Publication are considered to be published the day they are posted. If a serious error or ethical violation is identified before print publication, the Editor-in-Chief has the prerogative to print a revised manuscript or even withdraw a manuscript from print publication. While the rapid electronic version cannot be removed, it can be appropriately identified and stamped as a withdrawn or retracted manuscript.

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If the Editor-in-Chief, after any needed consultation with their Editorial Board, determines that there is reasonable cause to pro-

ceed with further investigation, he or she shall contact the institution with which the author is associated, and where the work was conducted. The Editor-in-Chief shall request to be informed of the progress and outcome of any inquiry or investigation, including any administrative or disciplinary action taken by the institution as a result. The pace of the inquiry or investigation and the frequency of progress reports will be set in reference to timelines established by the institution, if they exist, or by a mutually agreed upon timeline established between the institution and the Editor-in-Chief. In instances where the institution fails to meet expected timelines, the matter will be referred back to the Publications Committee for treatment as a special case. Similarly, in the rare event that the author against whom the allegation is made does not have an institutional affiliation, the matter will also be treated as a special case by the Publications Committee. A "special case" is a matter involving allegations of scientific misconduct in which the Publications Committee, rather than an author's institution, undertakes an investigation.

All parties involved are expected to cooperate fully to ensure fairness in the investigation. Authors are expected to cooperate by providing access to original data and laboratory notebooks or in other ways. The accused is considered innocent of wrongdoing until the evidence or investigation indicates otherwise. Any individual who brings forward allegations in good faith shall not be subjected to retaliation, however, allegations not brought in good faith, or maliciously motivated, may be subject to disciplinary action by the Council.

The outcome of the institutional investigation (or the special case investigation if no institution is involved) shall be reported to the Council of The Endocrine Society for a determination as to appropriate action, if any, including rejection of a manuscript or retraction of a publication. If the Council determines that, due to scientific misconduct, the validity or authoritativeness of a previously published article is in question, or the article contains a material error, then a correction or retraction must be published prominently in the journal in which the original report appeared and contain the full bibliographic reference to the original article. It shall be listed in the contents page and be prominently labeled, i.e., erratum or retraction.

The Council also reserves the right to reprimand the author, and co-authors, as it deems appropriate. Sanctions may include, but are not limited to, dismissal from the Society for a number of years, expulsion, written notification to other professional societies, institutions and funding agencies.

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advertisement it deems inappropriate. It accepts advertisements only if the advertisers warrant that the advertisement does not contravene legal requirements on trade descriptions, medicines, race relations, or sex discrimination.

Bibliography

In assembling these guidelines, the Publications Committee has relied on the following published reports:

1. Code of Ethics. The Endocrine Society (Chevy Chase, Maryland). *The Journal of Clinical Endocrinology & Metabolism*, *Endocrinology*, *Endocrine Reviews*, and *Molecular Endocrinology*, 2001. Code of Ethics of The Endocrine Society

2. ORI Handbook for Institutional Research Integrity Officers. February 1997. Office of Research Integrity, Public Health Service, Department of Health and Human Services (Washington DC)

3. Burman KD 1982 Hanging from the masthead: reflections on authorship. *Ann Intern Med* 97:602–605

4. Responsible Conduct Regarding Scientific Communication. Society for Neuroscience, 1996, 1997, 1998. <http://www.sfn.org>

5. Managing Allegations of Scientific Misconduct: A Guidance Document for Editors. January 2000. Office of Research Integrity, Office of Public Health and Science, U.S. Department of Health and Human Services (Washington, DC)

6. American College of Physicians Ethics Manual. American College of Physicians. *Ann Intern Med* 1984; 101: 129–187, 263–274

7. Rossner M, Yamada KM 2004 What's in a picture? The temptation of image manipulation *J Cell Biol* 166:11–15

8. Council of Science Editors 2006 CSE's White Paper on Promoting Integrity in Scientific Journal Publications

In addition, the Committee recommends reading the following:

1. Association of American Medical Colleges 1982 The maintenance of high ethical standards in the conduct of research.

2. Hugh EJ 1986 Guidelines on authorship of medical papers. *Ann Intern Med* 104:269.



Brief Summary: For complete details, please see full Prescribing Information.

INDICATIONS AND USAGE: BYETTA is indicated as adjunctive therapy to improve glycemic control in patients with type 2 diabetes mellitus who are taking metformin, a sulfonylurea, a thiazolidinedione, a combination of metformin and a sulfonylurea, or a combination of metformin and a thiazolidinedione, but have not achieved adequate glycemic control.

CONTRAINDICATIONS: BYETTA is contraindicated in patients with known hypersensitivity to exenatide or to any of the product components.

PRECAUTIONS: General—BYETTA is not a substitute for insulin in insulin-requiring patients. BYETTA should not be used in patients with type 1 diabetes or for the treatment of diabetic ketoacidosis.

Postmarketing cases of acute pancreatitis have been reported in patients treated with BYETTA. Patients should be informed that persistent severe abdominal pain, which may be accompanied by vomiting, is the hallmark symptom of acute pancreatitis. If pancreatitis is suspected, BYETTA and other potentially suspect drugs should be discontinued, confirmatory tests performed and appropriate treatment initiated. Resuming treatment with BYETTA is not recommended if pancreatitis is confirmed and an alternative etiology for the pancreatitis has not been identified.

Patients may develop anti-exenatide antibodies following treatment with BYETTA, consistent with the potentially immunogenic properties of protein and peptide pharmaceuticals. Patients receiving BYETTA should be observed for signs and symptoms of hypersensitivity reactions. In a small proportion of patients, the formation of anti-exenatide antibodies at high titers could result in failure to achieve adequate improvement in glycemic control.

The concurrent use of BYETTA with insulin, D-phenylalanine derivatives, meglitinides, or alpha-glucosidase inhibitors has not been studied.

BYETTA is not recommended for use in patients with end-stage renal disease or severe renal impairment (creatinine clearance <30 mL/min; see Pharmacokinetics, Special Populations). In patients with end-stage renal disease receiving dialysis, single doses of BYETTA 5 mcg were not well tolerated due to gastrointestinal side effects.

There have been rare, spontaneously reported events of altered renal function, including increased serum creatinine, renal impairment, worsened chronic renal failure and acute renal failure, sometimes requiring hemodialysis. Some of these events occurred in patients receiving one or more pharmacologic agents known to affect renal function/hydration status and/or in patients experiencing nausea, vomiting, and/or diarrhea, with or without dehydration. Concomitant agents included angiotensin converting enzyme inhibitors, nonsteroidal anti-inflammatory drugs, and diuretics. Reversibility of altered renal function has been observed with supportive treatment and discontinuation of potentially causative agents, including exenatide. Exenatide has not been found to be directly nephrotoxic in preclinical or clinical studies.

BYETTA has not been studied in patients with severe gastrointestinal disease, including gastroparesis. Its use is commonly associated with gastrointestinal adverse effects, including nausea, vomiting, and diarrhea. Therefore, the use of BYETTA is not recommended in patients with severe gastrointestinal disease.

Hypoglycemia—In the 30-week controlled clinical trials with BYETTA, a hypoglycemia episode was recorded as an adverse event if the patient reported symptoms associated with hypoglycemia with an accompanying blood glucose <60 mg/dL or if symptoms were reported without an accompanying blood glucose measurement. When BYETTA was used in combination with metformin, no increase in the incidence of hypoglycemia was observed. In contrast, when BYETTA was used in combination with a sulfonylurea, the incidence of hypoglycemia was increased over that of placebo in combination with a sulfonylurea. Therefore, patients receiving BYETTA in combination with a sulfonylurea may have an increased risk of hypoglycemia (Table 1).

Table 1: Incidence (%) of Hypoglycemia* by Concomitant Antidiabetic Therapy

	BYETTA			BYETTA			BYETTA		
	Placebo	5 mcg	10 mcg	Placebo	5 mcg	10 mcg	Placebo	5 mcg	10 mcg
	BID	BID	BID	BID	BID	BID	BID	BID	BID
	With Metformin			With a Sulfonylurea			With MET/SFU		
N	113	110	113	123	125	129	247	245	241
Hypoglycemia	5.3%	4.5%	5.3%	3.3%	14.4%	35.7%	12.6%	19.2%	27.8%

* In three 30-week placebo-controlled clinical trials. BYETTA and placebo were administered before the morning and evening meals. Abbreviations: BID, twice daily; MET/SFU, metformin and a sulfonylurea.

Most episodes of hypoglycemia were mild to moderate in intensity, and all resolved with oral administration of carbohydrate. To reduce the risk of hypoglycemia associated with the use of a sulfonylurea, reduction in the dose of sulfonylurea may be considered (see DOSAGE AND ADMINISTRATION). When used as add-on to a thiazolidinedione, with or without metformin, the incidence of symptomatic mild to moderate hypoglycemia with BYETTA was 11% compared to 7% with placebo.

BYETTA did not alter the counter-regulatory hormone responses to insulin-induced hypoglycemia in a randomized, double-blind, controlled study in healthy subjects.

Information for Patients—Patients should be informed of the potential risks of BYETTA. Patients should also be fully informed about self-management practices, including the importance of proper storage of BYETTA, injection technique, timing of dosage of BYETTA as well as concomitant oral drugs, adherence to meal planning, regular physical activity, periodic blood glucose monitoring and HbA_{1c} testing, recognition and management of hypoglycemia and hyperglycemia, and assessment for diabetes complications.

Patients should be advised to inform their physicians if they are pregnant or intend to become pregnant.

The risk of hypoglycemia is increased when BYETTA is used in combination with an agent that induces hypoglycemia, such as a sulfonylurea (see PRECAUTIONS, Hypoglycemia).

Patients should be advised that treatment with BYETTA may result in a reduction in appetite, food intake, and/or body weight, and that there is no need to modify the dosing regimen due to such effects. Treatment with BYETTA may also result in nausea (see ADVERSE REACTIONS). Patients should be informed that persistent severe abdominal pain, which may be accompanied by vomiting, is the hallmark symptom of acute pancreatitis and be instructed to contact their physician if this symptom occurs (see PRECAUTIONS).

Drug Interactions—The effect of BYETTA to slow gastric emptying may reduce the extent and rate of absorption of orally administered drugs. BYETTA should be used with caution in patients receiving oral medications that require rapid gastrointestinal absorption. For oral medications that are dependent on threshold concentrations for efficacy, such as contraceptives and antibiotics, patients should be advised to take those drugs at least 1 h before BYETTA

injection. If such drugs are to be administered with food, patients should be advised to take them with a meal or snack when BYETTA is not administered. The effect of BYETTA on the absorption and effectiveness of oral contraceptives has not been characterized.

Warfarin: Since market introduction there have been some spontaneously reported cases of increased INR with concomitant use of warfarin and BYETTA, sometimes associated with bleeding.

Carcinogenesis, Mutagenesis, Impairment of Fertility—A 104-week carcinogenicity study was conducted in male and female rats and benign thyroid C-cell adenomas were observed in female rats at all exenatide doses. The incidences in female rats were 8% and 5% in the two control groups and 14%, 11%, and 23% in the low-, medium-, and high-dose groups with systemic exposures of 5, 22, and 130 times, respectively, the human exposure resulting from the maximum recommended dose of 20 mcg/day.

In a 104-week carcinogenicity study in mice, no evidence of tumors was observed at doses up to 250 mcg/kg/day, a systemic exposure up to 95 times the human exposure resulting from the maximum recommended dose of 20 mcg/day.

Exenatide was not mutagenic or clastogenic, with or without metabolic activation, in the Ames bacterial mutagenicity assay or chromosomal aberration assay in Chinese hamster ovary cells.

Pregnancy—Pregnancy Category C—Exenatide has been shown to cause reduced fetal and neonatal growth, and skeletal effects in mice at systemic exposures 3 times the human exposure resulting from the maximum recommended dose of 20 mcg/day. Exenatide has been shown to cause skeletal effects in rabbits at systemic exposures 12 times the human exposure resulting from the maximum recommended dose of 20 mcg/day. There are no adequate and well-controlled studies in pregnant women. BYETTA should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

In pregnant mice an increased number of neonatal deaths were observed on postpartum days 2-4 in dams given 6 mcg/kg/day, a systemic exposure 3 times the human exposure resulting from the maximum recommended dose of 20 mcg/day.

Nursing Mothers—It is not known whether exenatide is excreted in human milk. Caution should be exercised when BYETTA is administered to a nursing woman.

Pediatric Use—Safety and effectiveness of BYETTA have not been established in pediatric patients.

Geriatric Use—BYETTA was studied in 282 patients 65 years of age or older and in 16 patients 75 years of age or older. No differences in safety or effectiveness were observed between these patients and younger patients.

ADVERSE REACTIONS: Use with metformin and/or a sulfonylurea—In the three 30-week controlled trials of BYETTA add-on to metformin and/or sulfonylurea, adverse events with an incidence ≥5% (excluding hypoglycemia; see Table 1) that occurred more frequently in patients treated with BYETTA (N = 963) vs placebo (N = 483) were: nausea (44% vs 18%), vomiting (13% vs 4%), diarrhea (13% vs 6%), feeling jittery (9% vs 4%), dizziness (9% vs 6%), headache (9% vs 6%), and dyspepsia (6% vs 3%).

The adverse events associated with BYETTA generally were mild to moderate in intensity. The most frequently reported adverse event, mild to moderate nausea, occurred in a dose-dependent fashion. With continued therapy, the frequency and severity decreased over time in most of the patients who initially experienced nausea. Adverse events reported in ≥1.0 to <5.0% of patients receiving BYETTA and reported more frequently than with placebo included asthenia (mostly reported as weakness), decreased appetite, gastroesophageal reflux disease, and hyperhidrosis. Patients in the extension studies at 52 weeks experienced similar types of adverse events observed in the 30-week controlled trials.

The incidence of withdrawal due to adverse events was 7% for BYETTA-treated patients and 3% for placebo-treated patients. The most common adverse events leading to withdrawal for BYETTA-treated patients were nausea (3% of patients) and vomiting (1%). For placebo-treated patients, <1% withdrew due to nausea and 0% due to vomiting.

Use with a thiazolidinedione—In the 16-week placebo-controlled study of BYETTA add-on to a thiazolidinedione, with or without metformin, the incidence and type of other adverse events observed were similar to those seen in the 30-week controlled clinical trials with metformin and/or a sulfonylurea. No serious adverse events were reported in the placebo arm. Two serious adverse events, namely chest pain (leading to withdrawal) and chronic hypersensitivity pneumonitis, were reported in the BYETTA arm.

The incidence of withdrawal due to adverse events was 16% (19/121) for BYETTA-treated patients and 2% (2/112) for placebo-treated patients. The most common adverse events leading to withdrawal for BYETTA-treated patients were nausea (9%) and vomiting (5%). For placebo-treated patients, <1% withdrew due to nausea. Chills (n = 4) and injection-site reactions (n = 2) occurred only in BYETTA-treated patients. The two patients who reported an injection-site reaction had high titers of anti-exenatide antibody.

Spontaneous Data—Since market introduction of BYETTA, the following additional adverse reactions have been reported. Because these events are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. **General:** injection-site reactions; dysgeusia; somnolence, INR increased with concomitant warfarin use (some reports associated with bleeding). **Allergy/Hypersensitivity:** generalized pruritus and/or urticaria, macular or papular rash, angioedema; rare reports of anaphylactic reaction. **Gastrointestinal:** nausea, vomiting, and/or diarrhea resulting in dehydration; abdominal distention, abdominal pain, eructation, constipation, flatulence, acute pancreatitis (see PRECAUTIONS). **Renal and Urinary Disorders:** altered renal function, including acute renal failure, worsened chronic renal failure, renal impairment, increased serum creatinine (see PRECAUTIONS).

Immunogenicity—Consistent with the potentially immunogenic properties of protein and peptide pharmaceuticals, patients may develop anti-exenatide antibodies following treatment with BYETTA.

OVERDOSAGE: Effects of an overdose include severe nausea, severe vomiting, and rapidly declining blood glucose concentrations. In the event of overdose, appropriate supportive treatment should be initiated according to the patient's clinical signs and symptoms.

DOSAGE AND ADMINISTRATION: BYETTA therapy should be initiated at 5 mcg per dose administered twice daily at any time within the 60-minute period before the morning and evening meals (or before the two main meals of the day, approximately 6 hours or more apart). BYETTA should not be administered after a meal. Based on clinical response, the dose of BYETTA can be increased to 10 mcg twice daily after 1 month of therapy. Each dose should be administered as a SC injection in the thigh, abdomen, or upper arm.


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BYETTA is indicated as adjunctive therapy to improve glycemic control in patients with type 2 diabetes mellitus who are taking metformin, a sulfonylurea, a thiazolidinedione, a combination of metformin and a sulfonylurea, or a combination of metformin and a thiazolidinedione, but have not achieved adequate glycemic control.

BYETTA is not indicated for the management of obesity, and weight change was a secondary endpoint in clinical trials.

Important Safety Information

BYETTA is not a substitute for insulin in insulin-requiring patients, and should not be used in patients with type 1 diabetes or for the treatment of diabetic ketoacidosis.

Patients should be observed for signs and symptoms of acute pancreatitis (persistent severe abdominal pain which may be accompanied by vomiting). If pancreatitis is suspected, BYETTA and other potentially suspect drugs should be discontinued.

Patients should be observed for signs and symptoms of hypersensitivity reactions.

BYETTA is not recommended for use in patients with end-stage renal disease, severe renal impairment, or severe gastrointestinal disease.

Patients should be observed for signs of altered renal function, including those who are taking concomitant agents known to affect renal function/hydration status.

Patients receiving BYETTA concomitantly with a sulfonylurea have an increased risk of hypoglycemia. To reduce the risk of hypoglycemia, clinicians may consider reducing the sulfonylurea dose.

The most common adverse events associated with BYETTA were nausea, vomiting, diarrhea, feeling jittery, dizziness, headache, and dyspepsia.

For additional safety information and other important prescribing considerations, please see the adjacent page for Brief Summary of Prescribing Information.

Visit www.ByettaHCP.com for more information.

*SDI data, March 2009.

References: 1. American Diabetes Association. Economic costs of diabetes in the U.S. in 2007. *Diabetes Care*. 2008;31(3):596-615. 2. Resnick HE, Foster GL, Bardsley J, Ratner RE. Achievement of American Diabetes Association clinical practice recommendations among U.S. adults with diabetes, 1999-2002: the National Health and Nutrition Examination Survey. *Diabetes Care*. 2006;29(5):531-537. 3. Bays HE, Chapman RH, Grandy S, for the SHIELD Investigators' Group. The relationship of body mass index to diabetes mellitus, hypertension and dyslipidaemia: comparison of data from two national surveys. *Int J Clin Pract*. 2007;61(5):737-747.

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