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expression in heat susceptible germ cells at early and late stages was observed as early as 2 hours preceding the DNA fragmentation in apoptotic germ cells occurring at 6 hours after heat exposure.
Conclusions: 1) Decreased testicular specific DDX4 expression disrupting RNA processing in translational regulation contributes to heat-induced germ cell apoptosis; 2) DDX4 is an early germ cell specific responder to heat stress in rat testis; 3) DDX4 may be a candidate target for male contraceptive development.

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MICROTUBULE ASSOCIATED SERINE/THREONINE KINASE 205 IS A NONRECEPTOR ACTIVATOR OF HETEROTRIMERIC G PROTIENS

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Purpose of Study: G-protein coupled receptors are essential for transducing signals from the extracellular environment of a cell and initiating an intracellular response via activation of heterotrimeric G proteins. Microtubule Associated Serine/Threonine Kinase 205 (MAST205) was previously discovered as a novel receptor-independent activator of heterotrimeric G proteins. Studies in Saccharomyces cerevisiae demonstrated that MAST205 interacts with the heteromeric G protein subunit Gαi. Based on these findings, we hypothesize that the kinase domain of MAST205 is responsible for interacting with Gαi.
Methods Used: Site-directed mutagenesis was used to create two kinase dead mutants by substituting methionine for each of two lysine residues in the kinase domain. The mutants were tested in a yeast strain that lacks a GPCR and contains a human Gαi subunit. A growth assay was used to study G-protein signaling in yeast transformed with both wild-type MAST205 and the kinase dead mutants. Yeast were grown on media lacking histidine, adenine, and containing varying concentrations of the histidine synthesis inhibitor 3-amino-1,2,4-triazole. MAST205 was fused to yellow fluorescent protein and expression in yeast confirmed by microscopy.
Summary of Results: Wild-type MAST205 signaled well (growth on 5mM 3-AT) whereas the kinase-dead mutants did not signal (no growth on 1 mM 3-AT) despite similar levels of expression. These findings demonstrate a role for the kinase activity of MAST205 in its ability to interact with Gαi.
Conclusions: The results are relevant because it has been well known that Gα interacts with Na/H exchanger. In addition, it has also been shown that MAST205 inhibits Na/H exchanger NHE 3. Future studies will focus on determining whether the MAST205-Gαi interaction contributes to inhibition of NHE 3 or Gαi-Na/H exchanger interactions.

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EVIDENCE THAT ENDOGENOUS INSULIN-LIKE GROWTH FACTOR-1 ATTENUATES ENDOTOXIN INDUCED CACHEXIA

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Purpose of Study: Cachexia - inflammatory wasting of lean muscle mass - causes morbidity and mortality in many common diseases, including cancer and advanced renal, hepatic and cardiac disease. Cachexia increases proteolysis while decreasing myogenesis leading to net muscle catabolism. Reduced serum insulin-like growth factor-1 (IGF-1) - a key anabolic hormone - may contribute to cachexia, since exogenously administered IGF-1 can reduce skeletal muscle loss. We hypothesized that endogenous muscle IGF-1 acting as a paracrine factor actively opposes cachexia by inhibiting proteolysis and up-regulating pro-myogenic anabolic signals.
Methods Used: Two experiments were performed utilizing subseptic endotoxin (100ng/g LPS ip) injections to induce cachexia in c57/B6 mice. In Experiment 1 animals were sacrificed 4, 22, and 44 hours after LPS treatment and tissues harvested. In Experiment 2 IGF-1 signaling was blocked during endotoxin-induced cachexia by administering an IGF-1 receptor neutralizing antibody (40mg/kg A12 ip) one day prior to LPS. Animals were sacrificed 22 hours after LPS treatment and tissues harvested. Body composition was analyzed using quantitative magnetic resonance (QMR) and skeletal muscle gene expression was evaluated using quantitative PCR of proteolytic (Atr-1, MuRF1), IGF-1, and myogenic MRF family (MyoD, MRF4, Myf5, Myogenin) genes.
Summary of Results: In Experiment 1, maximal induction of proteolytic gene expression occurred 22h after endotoxin administration. Skeletal muscle IGF-1 and MRF family (MyoD, Myf5, Myogenin) mRNA expression decreased significantly at 4h, but IGF-1 and MRF family (Myf5, MRF4) gene expression was significantly elevated at 22h. In Experiment 2, A12 significantly decreased the expression of MRF family genes (Myf5, Myogenin) while increasing expression of proteolytic genes.
Conclusions: These results demonstrate that endogenous IGF-1 signaling is activated in muscle during cachexia and attenuates proteolytic pathway gene expression while increasing expression of myogenic genes. Increasing muscle IGF-1 signaling may be a novel treatment paradigm for cachexia.

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Concurrent Session
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THERMOSENSITIVE BIODEGRADABLE POLYMERIC DRUG DELIVERY SYSTEM TO THE EYE

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Purpose of Study: Poly(N-isopropylacrylamide) (pNIPAAm) is a thermosensitive polymer that undergoes a reversible phase transition from liquid state at ambient temperature to solid at ≥32°C. The purpose of this study is to characterize an innovative polymer composition integrating pNIPAAm of a predefined molecular weight (MW) with biodegradable polyester as a platform for sustained drug release within the eye.
Methods Used: Polymer samples were synthesized from NIPAAm monomers and polycaprolactone (PCL) based macro-initiators, and characterized with rheometry. In vitro cytotoxicity and live-dead assays were applied to ensure polymer biocompatibility with NIH 3T3 fibroblast cultures. A 20% pNIPAAm solution was saturated with Norfloxacin antibiotics at 25°C, and drug release studies were performed at 37°C with release rates measured using UV-Vis spectrophotometry. In a pilot study, polymer-drug solutions were injected with a 25G needle into the sub-conjunctival space and in the anterior chamber of New Zealand white rabbit (NZW) eyes (n = 3), followed by monitoring of eye inflammation and drug release to the anterior chamber.
Summary of Results: Rheometrical measurements of the polymer's liquid phase transition showed a sharp increase of the storage modulus (G') near 32°C. Cytotoxicity assays demonstrated good tolerance of the cells after three days of growth over pre-heated polymer. Polymer was loaded with drug and injected with a syringe and 25G needle at 25°C, followed by solidification in less than 10 seconds at ≥32°C. This phase transition was reversible with lowered temperature. The in vitro drug studies showed an initial burst release within the first 48 hours, followed by steady drug release levels for 3 weeks. In vivo the polymer depots caused an initial moderate inflammation that was resolved after one day, and showed a burst release of drug within the first 4 hours and steady levels over the next 2 weeks.
Conclusions: Novel biodegradable thermosensitive polymers have been developed and initial studies demonstrate the feasibility for sustained drug delivery in the eye. Further development is indicated to optimize phase transition kinetics and delivery strategy suitable for clinical application.

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MORGELLONS DISEASE: ANALYSIS OF A POPULATION WITH CLINICALLY CONFIRMED MICROSCOPIC SUBCUTANEOUS FIBERS OF UNKNOWN ETIOLOGY

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Purpose of Study: Morgellons disease is a controversial illness in which patients complain of stinging, burning and biting sensations under the skin.

Unusual subcutaneous fibers are the unique objective finding. The etiology of Morgellons disease is unknown, and diagnostic criteria have yet to be established. Our goal was to identify prevalent symptoms in patients with clinically-confirmed subcutaneous fibers in order to develop a case definition for Morgellons disease.

Methods Used: Patients with subcutaneous fibers observed on physical examination (designated as the fiber group) were evaluated using a data extraction tool that measured clinical and demographic characteristics. The prevalence of symptoms common to the fiber group was then compared to the prevalence of these symptoms in patients with Lyme disease and no complaints of skin fibers.

Summary of Results: The fiber group consisted of 122 patients. Significant findings in this group were (1) an association with tick-borne diseases and hypothyroidism; (2) high numbers from two states, Texas and California; (3) high prevalence in middle-aged Caucasian women; and (4) an increased prevalence of smoking and substance abuse. Although depression was noted in 29% of the fiber patients, pre-existing delusional disease was not reported. After adjusting for non-specific symptoms, the most common symptoms reported in the fiber group were: crawling sensations under the skin; spontaneously-appearing, slow-healing lesions; hyperpigmented scars when lesions heal; intense pruritus; seed-like objects, black specks or "fuzz balls" in lesions or on intact skin; fine, thread-like fibers of varying colors in lesions and intact skin; lesions containing thick, tough, translucent fibers that are highly resistant to extraction; and a sensation of something trying to penetrate the skin from the inside out.

Conclusions: This study of the largest clinical cohort reported to date provides the basis for an accurate and clinically useful case definition for Morgellons disease.

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THE IMPACT OF METFORMIN ON VITAMIN B12 LEVELS IN SUBJECTS WITH TYPE 2 DIABETES

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Purpose of Study: The 2009 American Diabetes Association's Standards of Medical Care recommend metformin as a first-line therapy for Type 2 diabetes (DM2). Studies have suggested that metformin disturbs the Ca-dependent uptake of the B12-IF complex in the ileum resulting in B12 deficiency. With over 20 million people with DM2 in the U.S. and 40 million metformin prescriptions written in 2008 alone, a large population is at risk of B12 deficiency and it's sequelae including neurological deficits. The purpose of this pilot study is to characterize the relationship of metformin exposure to hypovitaminosis B12.

Methods Used: 50 veteran subjects, mean age 60 yrs [95% CI, 57–63] and mean time on metformin 5.4 yrs [95% CI, 0.4–12], were assessed during one study visit. Demographics, medical history and dietary information were obtained and BMI was calculated. Subjects were categorized by length of metformin exposure: < 2, 2–4, 4–6, and > 6 years. Subjects were also categorized by 'mg-years' (daily dosage x years on drug), which quantified their overall exposure to metformin, similar to the 'pack-years' notation used to quantify smoking exposure. These groups were then correlated with B12, Ca^{2+}, PO^{4-} and Mg^{2+} levels.

Summary of Results: In this pilot study, there was a significant difference in the primary endpoint of B12 levels in subjects taking metformin compared to a random sample of 500 veterans (349.1 pg/mL [95% CI, 306–393] vs. 530.9 pg/mL [95% CI, 489–573], P = .0065). 29% of subjects in this study had clinically low B12 values. This is significant compared to the B12 values of 500 random subjects, with only 11.5% having clinically low B12. Regression analysis of B12 and 'mg-years' was performed, indicating that 5.93% of B12 variability could be predicted by the length of metformin exposure, however due to the limited sample size this trend did not reach statistical significance (P = .099).

Conclusions: This study showed vitamin B12 levels in our study population of veterans with DM2 treated with metformin were significantly lower compared to a random sample of the veteran population. The results of this pilot study indicate a need for further research on the detrimental effects of metformin on B12 levels. This information is essential and can provide a wide impact on the care of people with DM2.

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US WHITE VERSUS INDIAN-BASED BONE MINERAL DENSITY REFERENCE STANDARDS FOR THE DIAGNOSIS OF OSTEOPOROSIS AND OSTEOPENIA AMONG SOUTH-ASIAN INDIANS

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Purpose of Study: The relationship between bone mineral density (BMD) and fracture risk is not well defined in non-white populations. There is no established reference standard for BMD for South Asians. This study assesses whether choosing between BMD reference standards based on US white, North Indian or South Indian populations affects the diagnosis of osteoporosis and osteopenia in South Asian Indians residing in the US.

Methods Used: Dual energy x-ray absorptiometry (DXA) was used to measure BMD at total hip and lumbar spine in 150 US-based South Asian Indians. We compared World Health Organization (WHO) BMD category assignment resulting from the use of US white, North Indian and South Indian reference values, and assessed the level of agreement among the standards using the proportions of subjects reclassified. We employed both North and South Indian standards because of known genotypic and phenotypic differences between these groups.

Summary of Results: US-White, North Indian and South Indian BMD reference standards differed in their propensity to diagnose osteoporosis and osteopenia in US-based South Asian Indians. For total hip, using standards based on South Indians and North Indians instead of US whites resulted in the reclassification of 19% (95% CI = 14–25%) and 13% (95% CI = 7–18%) of subjects to an improved BMD category respectively. Switching from US white to South Indian standards resulted in the reclassification of 40% (95% CI = 32–48%) of subjects to better BMD categories at the hip. We were unable to asses the effect of employing North Indian references standards at the lumbar spine due to the unavailability of spine reference data from this region.

Conclusions: Choice of BMD reference standard may affect the diagnosis and treatment of osteoporosis in US-based South Asian Indians, and should be taken into consideration by clinicians. Prospective data correlating BMD and fracture risk are needed to establish definitive diagnostic thresholds in this population.

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HIGHER A1c AND BMI ARE ASSOCIATED WITH WORSE DIABETIC DYSLIPIDEMIA IN A VA POPULATION

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Purpose of Study: Diabetic dyslipidemia is characterized by low HDL, high triglycerides(TG),small and dense LDL. Lipid therapy is critical for CVD risk reduction in diabetic patients. Because calculated LDL is limited in hypertriglyceridemia, non-HDL is recommended as an alternative treatment goal at TG > 200 mg/dl. In addition, non-HDL, which includes LDL, VLDL and IDL, is a surrogate for highly artherogenic apoB. Previous studies indicated that glycemic and weight control may affect different lipid components. However, the results varied among studies; the correlation between weight; glycemia and lipid pattern remains to be clarified.

We sought to determine the association between BMI; A1c and lipid profiles in type 2 diabetic outpatients.

Methods Used: Retrospective chart review was conducted on 4569 diabetic outpatients. Data on age, sex, BMI, A1c, fasting lipid panel and medications were collected. ANOVA was used for statistical analysis.

Summary of Results: More than 98% of the patients were male, with the following mean values: age 69.5 years; total cholesterol(TC) 159 mg/dl; HDL 37 mg/dl; LDL 90 mg/dl; TG 161 mg/dl; non-HDL 122 mg/dl; A1c 6.95 %; BMI 31.6 kg/m2. We grouped patients by NCEP TG classification with mean values in the table. LDL values were not calculated in 30 cases with TG >= 400mg/dl.

Conclusions: We found a statistically significant direct correlation between BMI; A1c values and the level of TG. Elevated TG was significantly associated with higher TC and non-HDL, but lower HDL. Calculated LDL