Krmpotić A, Hasan M, Loewendorf A, Saulig T, Halenius A, Lenac T, et al. NK cell activation through the NKG2D ligand MULT-1 is selectively prevented by the glycoprotein encoded by mouse cytomegalovirus gene m145. J Exp Med. 2005;201:211-20.

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The NK cell-activating receptor NKG2D interacts with three different cellular ligands, all of which are regulated by mouse cytomegalovirus (MCMV). The authors set out to define the viral gene product regulating murine UL16-binding protein-like transcript (MULT)-1, a newly described NKG2D ligand. They found that MCMV infection strongly induces MULT-1 gene expression, but surface expression of this glycoprotein is nevertheless completely abolished by the virus. Screening a panel of MCMV deletion mutants defined the gene m145 as the viral regulator of MULT-1. The MCMV m145-encoded glycoprotein turned out to be necessary and sufficient to regulate MULT-1 by preventing plasma membrane residence of MULT-1. The importance of MULT-1 in NK cell regulation *in vivo* was confirmed by the attenuating effect of the m145 deletion that was lifted after NK cell depletion. These findings underline the significance of escaping MULT-1/NKG2D signaling for viral survival and maintenance.

Peričić D, Jazvinšćak Jembrek M, Švob Štrac D, Lazić J, Špoljarić IR. Enhancement of benzodiazepine binding sites following chronic treatment with flumazenil. Eur J Pharmacol. 2005;507:7-13.

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The aim of this study was to improve the knowledge of the mechanisms leading to adaptive changes in gamma-aminobutyric acid$_A$ ($GABA_A$) receptors following chronic drug treatment. Exposure (48 h) of human embryonic kidney (HEK 293) cells stably expressing recombinant alpha1beta2gamma2S $GABA_A$ receptors to the antagonist of benzodiazepine binding sites, flumazenil (5 μM), enhanced the maximum number (B_{max}) and the equilibrium dissociation constant (K_d) of [^{3}H]flunitrazepam binding sites. The flumazenil-induced enhancement in B_{max} was potentiated by GABA (50 μM) and reduced by the $GABA_A$ receptor antagonist, bicuculline (100 μM). Flumazenil-induced enhancement in K_d was affected by neither of these treatments. GABA (50 μM) enhanced the density of [^{3}H]flunitrazepam binding sites, and this enhancement was greater in the presence of diazepam (1 μM). The results suggest that chronic flumazenil treatment up-regulates in a bicuculline-sensitive manner benzodiazepine binding sites at stably expressed $GABA_A$ receptors.

Ćulić V, Eterović D, Mirić D. Meta-analysis of possible external triggers of acute myocardial infarction. Int J Cardiol. 2005;99:1-8.

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Although it is well known that the acute myocardial infarction can be triggered by events such as physical activity, emotional stress, sexual activity or eating, the observed frequencies of these events preceding the onset of myocardial infarction vary between published reports. A meta-analysis of 17 seldom population-based studies that included data on frequency of external triggers or onsets during sleep was performed. Of the 10519 patients, heavy physical activity was recorded before the onset of myocardial infarction in 6.1%, whereas mild-to-moderate physical activity was recorded in 28.6% of 7517 patients. Eating preceded the onset in 8.2% of 4785 patients, various kinds of emotional stress in 6.8% of 2565 (particularly anger in 2.1% of 2283), meteorologic stress in 3.7% of 3371, and sexual activity in 1.1% of 3406 patients. Out of 11778 patients, 20.7% had infarction onset during sleep. Triggers in general (OR = 1.45, 95% CI = 1.21-1.76; $p < 0.0001$), heavy physical activity (OR = 6.21, 95% CI = 3.77-10.23; $p < 0.0001$) and eating (OR = 1.70, 95% CI = 1.14-2.53; $p = 0.0008$) were more likely to precede the infarction onset in men while women were more likely to report emotional stress (OR = 0.66, 95% CI = 0.50-0.86; $p = 0.002$). This analysis defines the occurrence of possible external triggers before the onset of myocardial infarction in general population, but their actual contribution to the very onset is somewhat less frequent. Future investigation should identify other eventual triggers unrecognized as yet, asses the risk of triggering myocardial infarction among patients with defined levels of ische-

mic heart disease and further elucidate the pathophysiologic mechanisms of gender differences and beneficial effect of habitual physical activity.

Glamočlija V, Vilović K, Saraga-Babić M, Baranović A, Sapunar D. Apoptosis and active caspase-3 expression in human granulosa cells. Fertil Steril. 2005;83:426-31.

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The aim of this study was To document the expression of activated forms of caspase-3 in human granulosa cells. Ovarian tissues were obtained from women undergoing hysterectomy/ ovariectomy for benign conditions and human granulosa cells were obtained from women undergoing oocyte retrieval for IVF interventions. Immunostaining of tissue sections and cell smears was done using antibody to active caspase-3 and terminal deoxynucleotidyl transferase (TdT) assay (TUNEL) for detection of internucleosomal DNA fragmentation. In human ovarian tissue, no apoptosis was observed in primordial and primary follicles. Apoptosis in granulosa cells was detected only in atretic antral follicles. Granulosa cells classified as apoptotic on the basis of their morphologic features contained a single condensed nucleus, multiple nuclear fragments, or apoptotic bodies. All apoptotic granulosa cells expressed active caspase-3, but only few contained fragmented DNA detected with the TUNEL method. The expression of active caspase-3 was also demonstrated in human granulosa cells of preovulatory follicles obtained from patients undergoing IVF. In conclusion, caspase-3 dependent apoptosis occurs in human granulosa cells and activates when follicles begin to leave the resting pool. After initial formation of the antrum, activation of caspase-3 is a normal physiologic process of the follicle during atresia and luteinization. Higher numbers of granulosa cells positive with caspase-3 than cells positive with TUNEL suggest an earlier activation of caspase-3 compared with the DNA fragmentation detected by TUNEL assay and also a longer detection period of caspase-3 than DNA fragmentation in apoptotic granulosa cells.

Lukić IK, Grčević D, Kovačić N, Katavić V, Ivčević S, Kalajzić I, et al. Alteration of newly induced endochondral bone formation in adult mice without tumour necrosis factor receptor 1. Clin Exp Immunol. 2005;139:236-44.

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Tumour necrosis factor (TNF)-alpha, a major proinflammatory cytokine, exerts its role on bone cells through two receptors (TNFR1 and TNFR2). TNFR1, but not TNFR2, is expressed by osteoblasts and its function in bone formation in vivo is not fully understood. We compared in vivo new bone formation in TNFR1-deficient ($TNFR1^{-/-}$) mice and wild-type mice, using two models of bone formation: intramembranous ossification following tibial marrow ablation and endochondral ossification induced by bone morphogenetic protein (BMP)-2. Intramembranous osteogenesis in $TNFR1^{-/-}$ mice did not differ from the wild-type mice either in histomorphometric parameters or mRNA expression of bone-related markers and inflammatory cytokines. During endochondral osteogenesis, $TNFR1^{-/-}$ mice formed more cartilage (at post-implantation day 9), followed by more bone and bone marrow (at day 12). mRNAs for BMP-2, -4 and -7 were increased during the endochondral differentiation sequence in $TNFR1^{-/-}$ mice. The expression of receptor activator of NF-kappa B ligand (RANKL) and receptor activator of NF-kappa B (RANK), as assessed by quantitative reverse transcription polymerase chain reaction (RT-PCR), was also increased significantly during endochondral ossification in $TNFR1^{-/-}$ mice. In conclusion, signalling through the TNFR1 seems to be a negative regulator of new tissue formation during endochondral but not intramembranous osteogenesis in an adult organism. BMPs and RANKL and its receptor RANK may be involved in the change of local environment in the absence of TNFR1 signalling.

Sobočanec S, Abalog T, Šverko V, Marotti T. Met-enkephalin modulation of age-related changes in red cell antioxidant status. Physiol Res. 2005;54:97-104.

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Opioid peptides have been recognized as modulators of reactive oxygen species (ROS) in mouse macrophages and human neutrophils. Since the effect cannot be ascribed to its direct scavenger properties, the authors tested the hypothesis that methionine-enkephalin (MENK) modulates ROS by alteration of antioxidant enzyme activity (AOE). For this purpose superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX) are measured in red blood cells of 1, 4, 10, and 18-month-old CBA mice of both sexes injected with 10 mg/kg MENK. The results indicate that MENK-affected antioxidant enzyme activity of red blood cells is age- but not sex-related. The most abundant effects were observed at the reproductive stage. Increased sensitivity to oxidative stress by opioid peptides was in both sexes mainly due to increased SOD activity followed by GPX decrease. Thus, the damage ascribed to opioid peptides might be, at least partly, ascribed to deleterious effects of accumulated hydrogen peroxide (H_2O_2).