Gruppo di quesiti n.1

- Indicare la modalità attraverso cui ottenere la coltivazione di cellule a bassa, media ed elevata concentrazione di glucosio, e potenziali applicazioni.
- Descrivere le metodiche di distacco delle cellule con fenotipo aderente.

Conoscenza lingua inglese: tratto da: *Diabetes* 2023;72(12):1751–1765

https://doi.org/10.2337/db22-1033

- Caspases are cysteine-aspartic proteases that were initially discovered to play a role in apoptosis. However, caspase 8, in particular, also has additional nonapoptotic roles, such as in inflammation. Adipocyte cell death and inflammation are hypothesized to be initiating pathogenic factors in type 2 diabetes. Here, we examined the pleiotropic role of caspase 8 in adipocytes and obesity-associated insulin resistance. Caspase 8 expression was increased in adipocytes from mice and humans with obesity and insulin resistance. Treatment of 3T3-L1 adipocytes with caspase 8 inhibitor Z-IETD-FMK decreased both death receptor–mediated signaling and targets of nuclear factor κ-light-chain-enhancer of activated B (NF-κB) signaling. These data demonstrate an important role for caspase 8 in mediating adipocyte cell death and inflammation to regulate glucose and energy homeostasis.

Gruppo di quesiti n.2

- Descrivere come si esegue una marcatura per la lettura al citofluorimetro.
- Descrivere i principi di estrazione dell' RNA da campione cellulare e l'analisi in real-time.

Conoscenza lingua inglese: tratto da: *Diabetes* 2023;72(12):1795–1808

https://doi.org/10.2337/db23-0032

There is clinical evidence that increased urinary serine proteases are associated with the disease severity in the setting of diabetic nephropathy (DN). Elevation of serine proteases may mediate $[Ca^{2+}]_i$ dynamics in podocytes through the protease-activated receptors (PARs) pathway, including associated activation of nonspecific cation channels. Cultured human podocytes and freshly isolated glomeruli were used for fluorescence and immunohistochemistry stainings, calcium imaging, Western blot analysis, scanning ion conductance microscopy, and patch clamp analysis. Goto-Kakizaki, Wistar, type 2 DN (T2DN), and a novel PAR1 knockout on T2DN rat background rats were used to test the importance of PAR1-mediated signaling in DN settings. We found that PAR1 activation increases $[Ca^{2+}]_i$ via TRPC6 channels

Gruppo di quesiti n.3

- Descrivere vantaggi e svantaggi della metodica ELISA per la quantificazione di proteine.
- Descrivere i principali saggi per la determinazione della morte cellulare.

Conoscenza lingua inglese: tratto da: *Diabetes* 2023;72(12):1820–1834

https://doi.org/10.2337/db23-0171

Many people living with diabetes also have nonalcoholic fatty liver disease (NAFLD). Interleukin-6 (IL-6) is involved in both diseases, interacting with both membrane-bound (classical) and circulating (trans-signaling) soluble receptors. We investigated whether secretion of IL-6 trans-signaling coreceptors are altered in NAFLD by diabetes and whether this might associate with the severity of fatty liver disease. Secretion patterns were investigated with use of human hepatocyte, stellate, and monocyte cell lines. Associations with liver pathology were investigated in two patient cohorts: *1*) biopsy-confirmed steatohepatitis and *2*) class 3 obesity. We found that exposure of stellate cells to high glucose and palmitate increased IL-6 and soluble gp130 (sgp130) secretion.