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Genotoxic and inflammatory responses of human bronchial epithelial cells to diesel and biodiesel exhaust.

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Background

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- There is an increasing application of renewable fuel sources (such as biodiesels) into the global fuel chain.
  - Current EU mandate for member states to use 10 % renewable energy in transport will however be scrapped after 2020.

- The increase is being driven by a number of environmental and health factors – focus to reduce the levels of toxic emissions (i.e. CO_2, NO_x, PAHs).

- Alongside this there is a growing interest in studying the adverse health effects of human exposure to contemporary diesel exhausts.

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- In a human exposure chamber study (Unosson et al., 2017, EHP in press) subjects were exposed to contemporary low-sulphur diesel (LSDE), RME100 and a blend of the two (RME30).
  - 70/30 LSDE/RME chosen as considered most likely blend for future.

- **Composition**: RME exhausts contained an increased number of smaller diameter particles (mean ⌀ 30-80 nm), lower EC and fewer PAHs.

- **Results overview**: RME30 and RME100 caused similar cardiovascular and respiratory symptoms in humans compared to LSDE.

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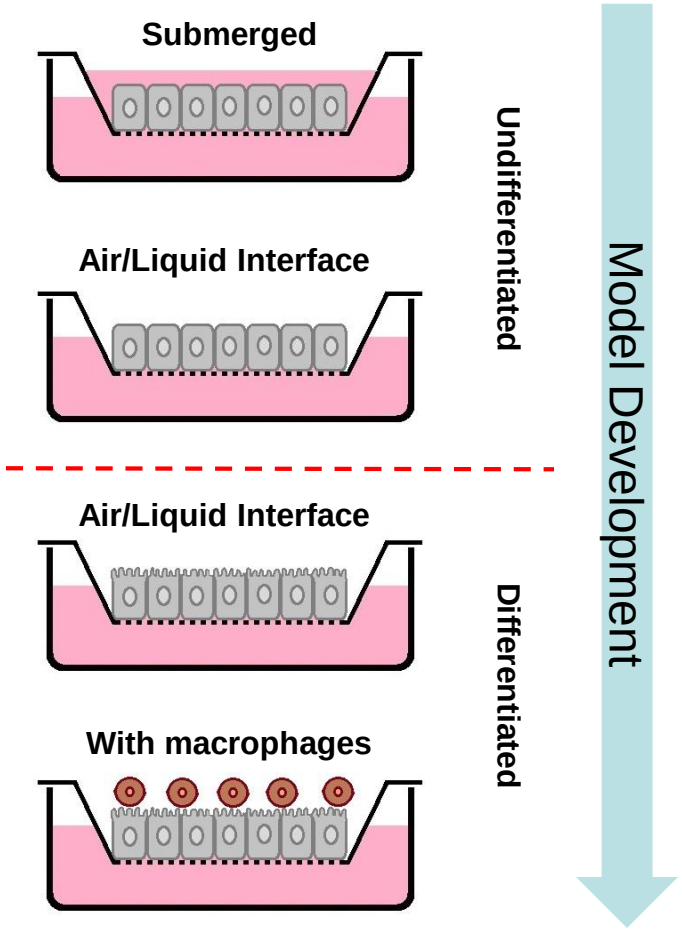
- My primary research involves development of improved *in vitro* lung cell models for toxicological testing.
- Primary human lungs cells are cultured on Corning Transwell inserts.
- Aim is to build up multiple cell types to increase complexity and ideally be more representative of the human airways.



Background

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- **Research question:** What are the *in vitro* effects of exposure of primary human lung cells to the contemporary LSDE and RME30 exhaust samples?

- In March 2017 I received LSDE and RME30 exhaust that had been collected by direct impinging into water (2.47 and 1.89 mg/ml, resp.).

- Today I will present some of our preliminary investigations in submerged primary human lung cells exposed to LSDE and RME30 exhaust.

Cellular assays

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- Primary human airway epithelial cells (hAEC) were obtained from bronchial biopsies of healthy non-smokers (Epithelix Sàrl, Geneva).

- Cells were exposed to 1.25 – 20 µg/ml exhaust (diluted in medium).
  - Range chosen from blood cell exposures (Ian Mudway group, KCL).
  - Exposure to SRM 2975 also to compare with older diesel exhaust.

- Endpoints studied:
  - Comet assay for DNA damage at 24/48 hrs.
  - Real-time PCR for mRNA levels of *CSF2, IL8 and TNF* at 1 – 48 hrs.
  - Western blot for phosphorylation of JNK, p38 and NFκB at 6 hrs.

Cytotoxicity

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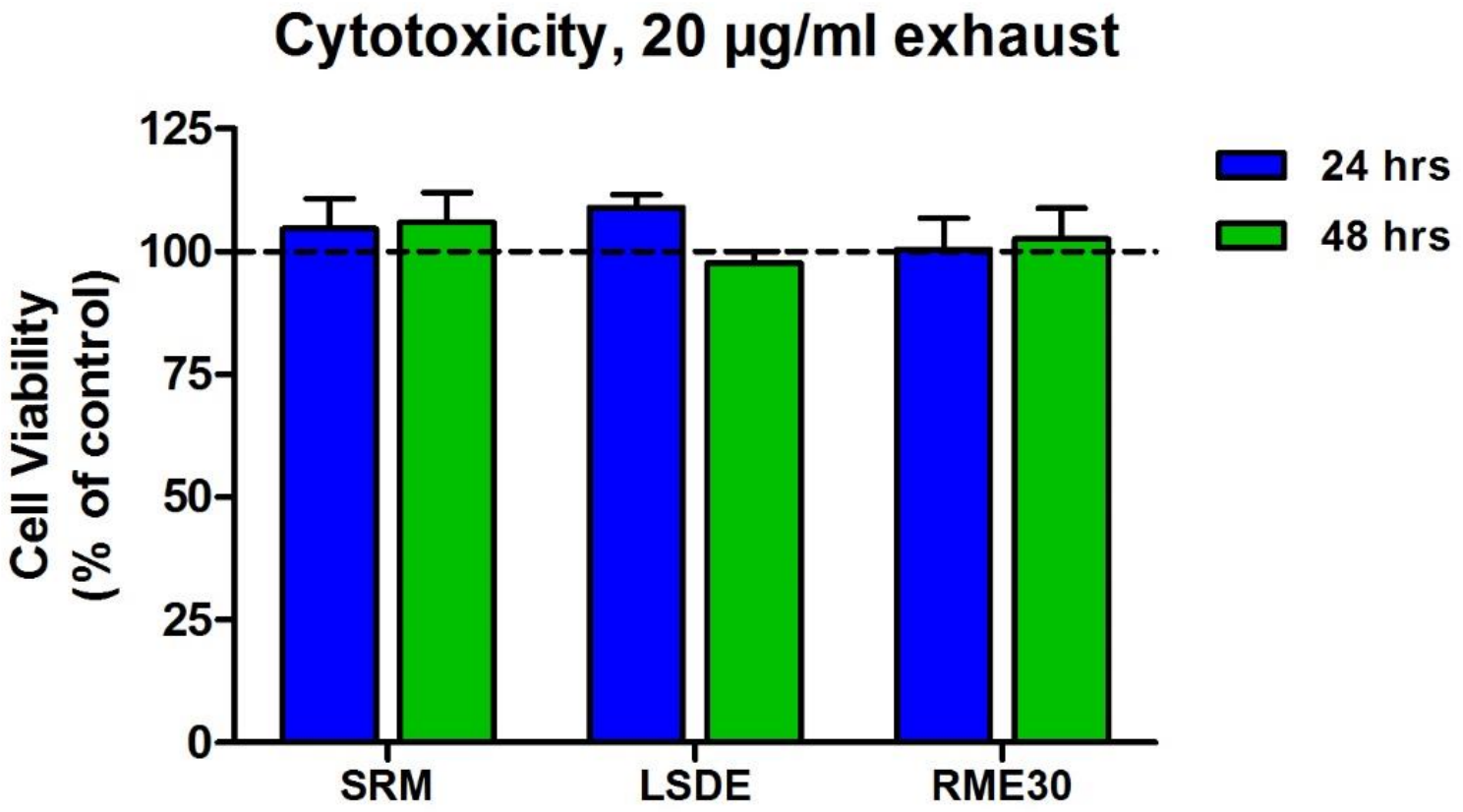

** = p<0.05 versus control, # = p<0.05 versus SRM*

DNA damage (comet)

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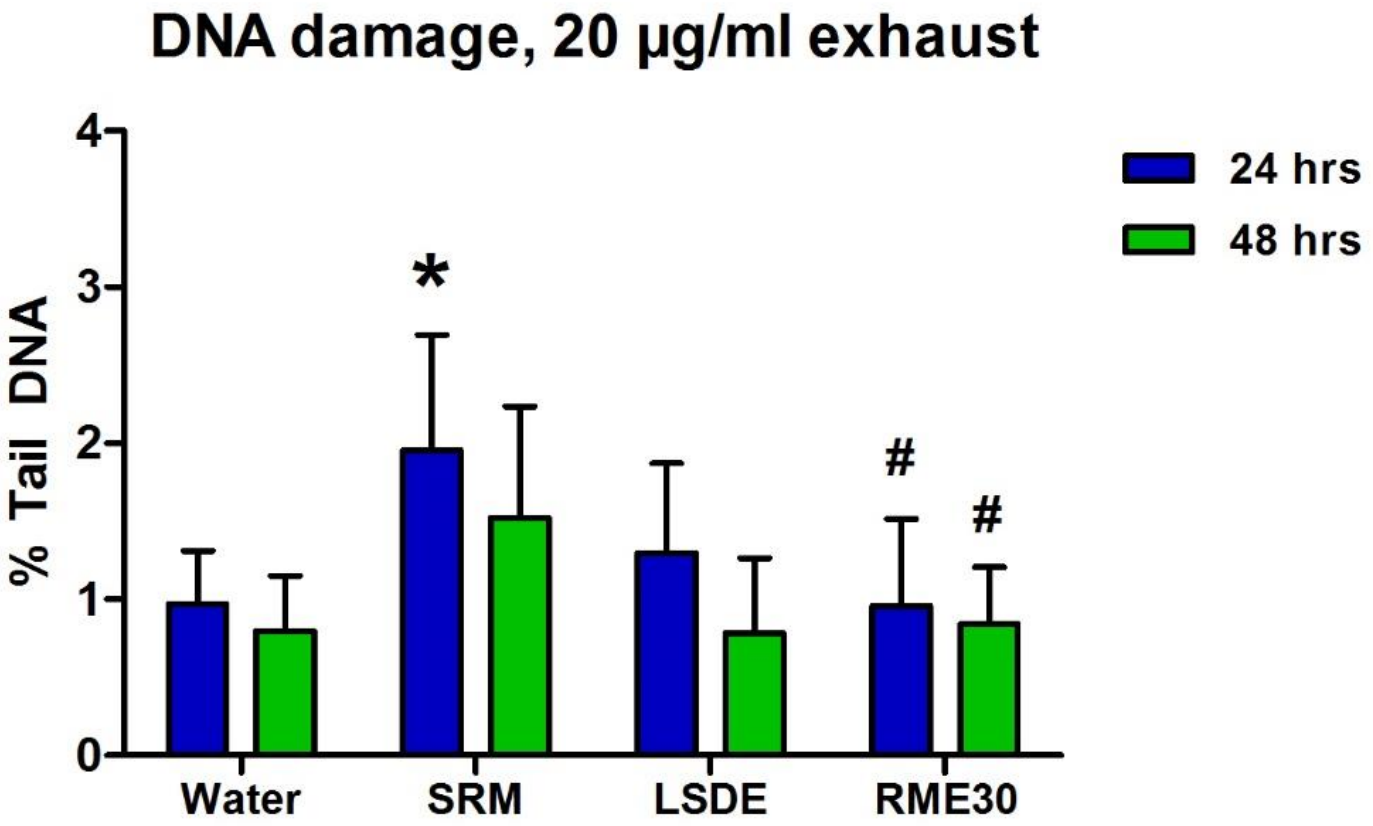

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Real Time PCR for *CSF2*

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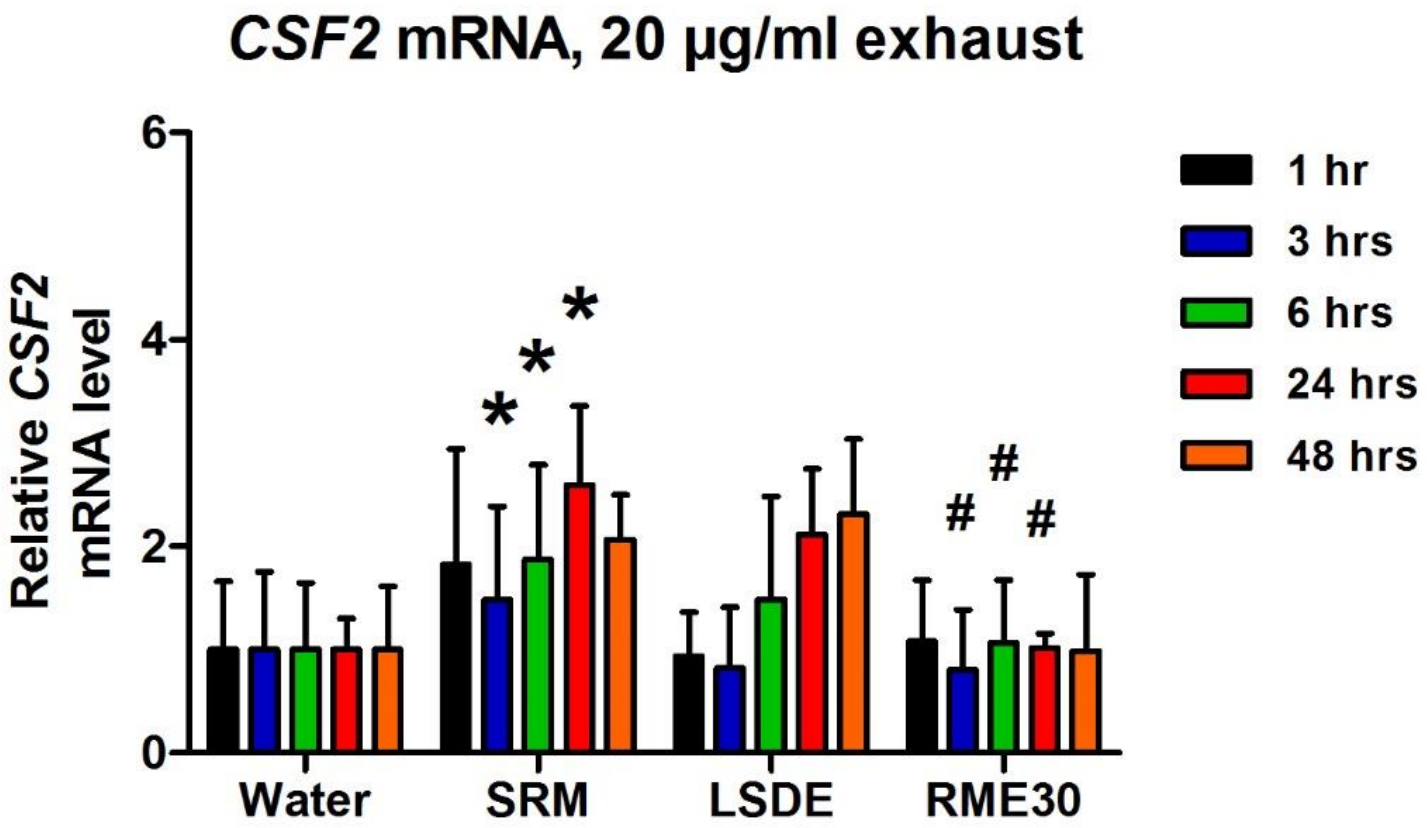
- Encodes GM-CSF cytokine, involved in recruitment of immune cells.



** = p<0.05 versus control, # = p<0.05 versus SRM*

Real Time PCR for *IL-8*

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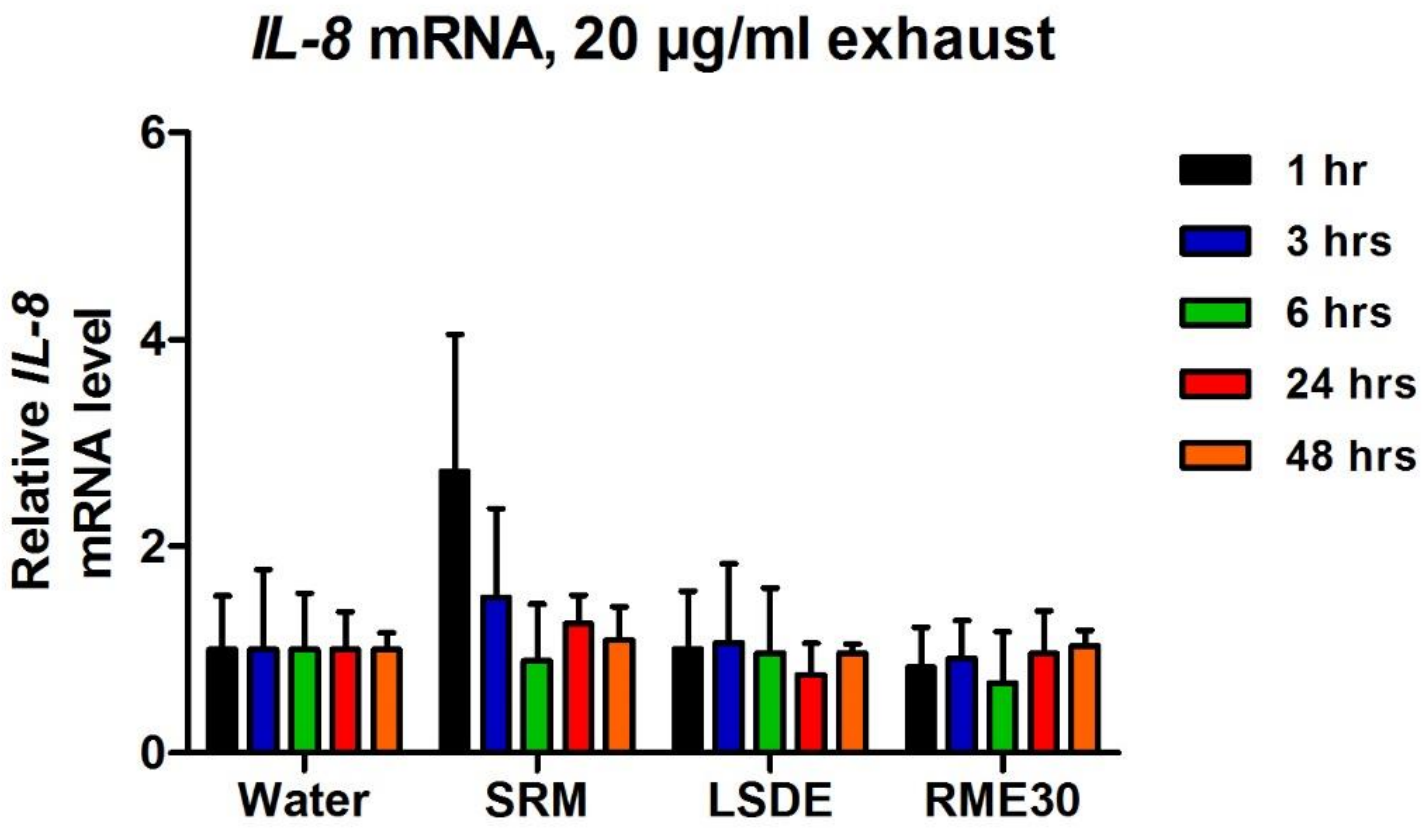
- Encodes IL-8 cytokine, involved in acute phase inflammatory response.



** = p<0.05 versus control, # = p<0.05 versus SRM*

Real Time PCR for *TNF*

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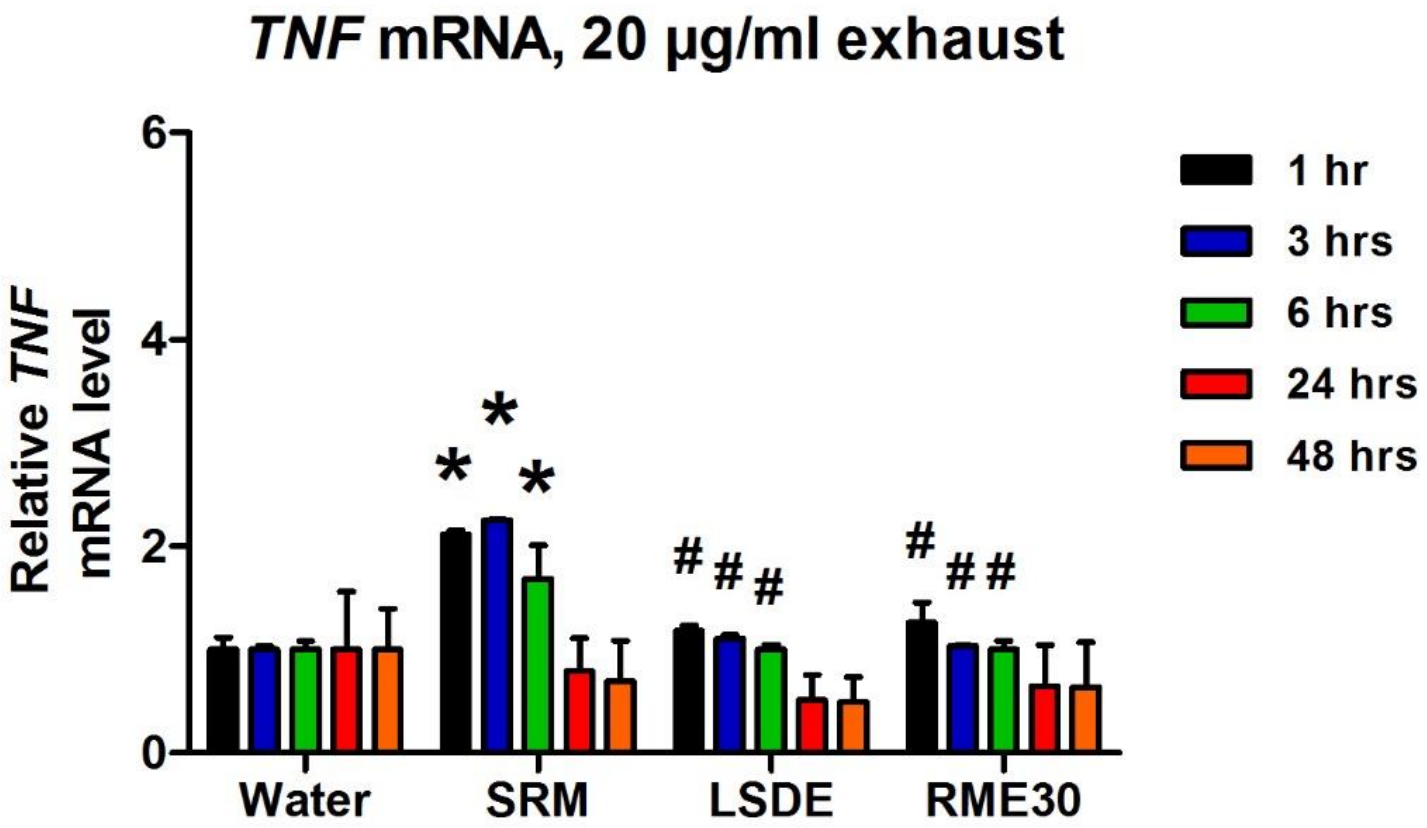
- Encodes TNFα cytokine, involved in acute phase inflammatory response.



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WB for stress signalling

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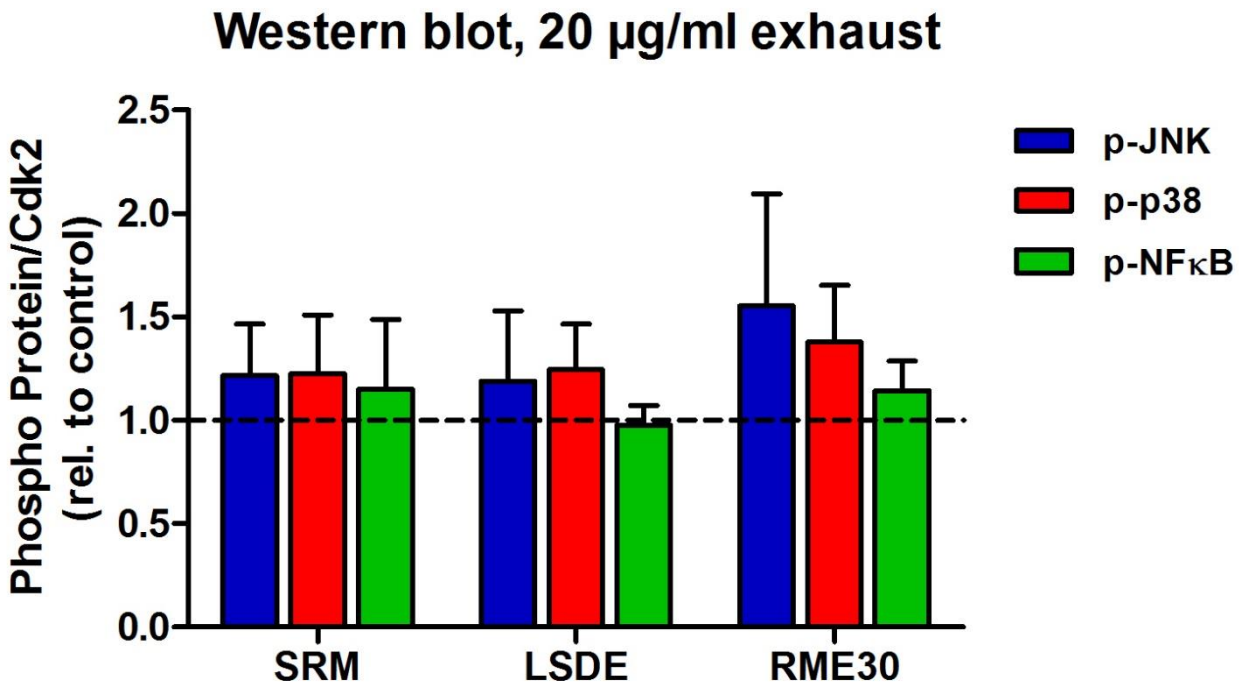
- Three proteins involved in a number of different responses to stimuli that cause cell stress including DNA transcription and cytokine production.



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Summary

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- Under the conditions tested, there was no difference in the *in vitro* response to RME30 compared to LSDE.

- Contemporary exhausts RME30 and LSDE elicited different cellular responses to the older diesel exhaust SRM 2975.

- Lack of an observed effect *in vitro* does not correlate with respiratory effects seen in human chamber exposures.

- Work is ongoing to understand effects of exposure to higher exhaust concentrations and repeat-dose exposures.

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