PROTECTIVE EFFECTS OF GREEN SHELLED MUSSELS IN OSTEOARTHRITIS

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Background: Obesity-induced chronic inflammation is associated with metabolic syndrome, and often leads to the development of osteoarthritis. In osteoarthritis, inflammatory cells act on both bone cells and cartilage cells to cause destruction of the joint. Green shelled mussels (GSM), a seafood native to New Zealand, have been shown to inhibit inflammation and reduce pain in the joints of animals with arthritis.

Methods: Female Sprague-Dawley rats (N>10 per group) were fed a normal control (CON) or high-fat/high-sugar (HFHS) diet with or without the inclusion of freeze-dried whole GSM from age 3 months to 7 months. Rats were assessed for serum levels of CTX-2, a biomarker for cartilage degradation. In addition, GSM extracts were used in vitro to treat undifferentiated bone cells (RAW 264.7 cells) and assessed for their ability to prevent the cells from differentiating into bone-destroying osteoclasts and producing tartrate-resistant acid phosphatase (TRAP).

Results: Unsurprisingly, rats fed a HSHS diet gained more weight and produced more CTX-2 compared to those fed a CON diet. However, the inclusion of GSM in both diets resulted in a reduction of cartilage degradation: CTX-2 levels in rats fed CON vs CON+GSM were 194+26 vs 161+25 pg/mL, while CTX-2 levels in rats fed HFHS vs HFHS+GSM were 241+31 vs 151+19 pg/mL ($p = 0.02$). In vitro, a non-polar lipid extract of GSM significantly reduced osteoclast differentiation in a dose-dependent manner, with treatment at 20 µg/mL reducing differentiation by 80% and TRAP production by 85%, whereas a polar lipid extract had no effect.

Conclusions: Dietary GSM significantly reduces the development of joint osteoarthritis caused by diet-induced metabolic syndrome in obese rats. Non-polar lipids in GSM in vitro significantly reduces the development of bone-resorbing osteoclast cells. Inclusion of GSM in the human diet is likely to protect both joint and bone health.

METHODS

ANIMAL MODEL

The influence of feeding a diet supplemented with freeze-dried green-shelled mussels (GSM) on joint health was evaluated in normal-weight rats and in obese rats that were likely to have developed obesity-induced osteoarthritis. Joint health was assessed by measuring CTX-2, a biomarker of cartilage breakdown. Female Sprague-Dawley rats from age 3 mo - 7 mo were fed one of 4 diets (N=12 per group): control (CON); CON+GSM; high fat + high sugar (HFHS); HFHS+GSM. Body weight was evaluated weekly. Plasma CTX-2 in blood taken at age 7 mo was measured by competitive ELISA in duplicate.

GSM FRACTIONATION

Lipid extraction

S 1 (Total lipid)
GSM meat was homogenized and extracted by Soxhlet apparatus. The total lipid was harvested.

Silica column separated lipid fractions

All lipid fractions were treated with lipases at 40ºC for 5 hours, then kept at -20°C.

S 2 (Polar lipid)
Mobile phase: run gradient 15% -100% ether.

S 3 (Non-polar lipid)
Mobile phase: hexane and run gradient to 15% ether.

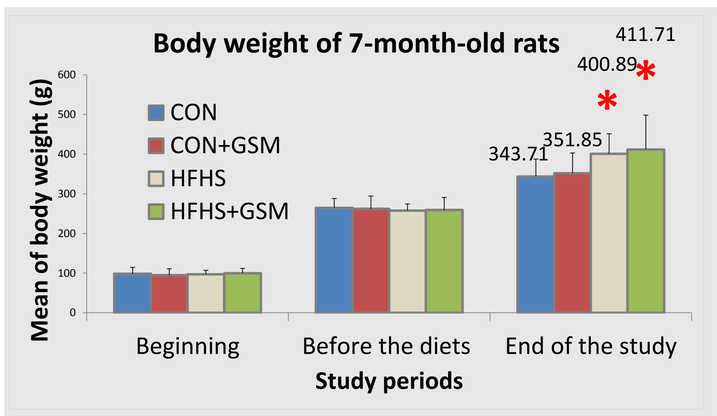
CELL CULTURE

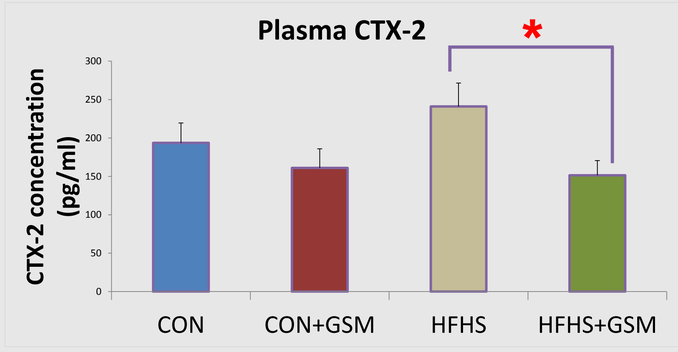
Cytotoxicity assay : Pre-osteoclast RAW 264.7 cells were cultured with the lipid extracts for or 6 days. MTT assay and morphology were used to measure the cell viability and to identify non-cytotoxic concentrations of the three lipid extracts.
Osteoclast differentiation assay : RAW 264.7 cells were seeded at $1.5X10^4$ cells/ml into 24-well plates. Lipid extracts were introduced on day 1 with RANKL (15 ng/ml) in triplicate. Media were replaced at day 4 and then collected at day 6. TRAP enzymatic activity was measured indirectly by p-nitrophenol,. The remaining cell monolayer was processed immediately for TRAP cell staining to visually identify large, multi-nucleated osteoclasts.

RESULTS: ANIMAL MODEL

Rats aged 3 mo were randomised by body weight (“beginning”). After 2 mo on chow, body weights did not vary (“before the diets”). After 4 mo on test diets (“end of study”), rats on HFHS diets weighed significantly more than those on CON (*p<0.05 by ANOVA. Obese rats at age 7 mo also had increased body length (far right photo).

Cartilage degradation was assessed by measuring plasma collagen type 2 (CTX-2). CTX-2 was elevated in HFHS rats fed compared to CON. Adding GSM to HFHS significantly reduced CTX-2 compared to HFHS alone ($p < 0.05$, Student t-test).



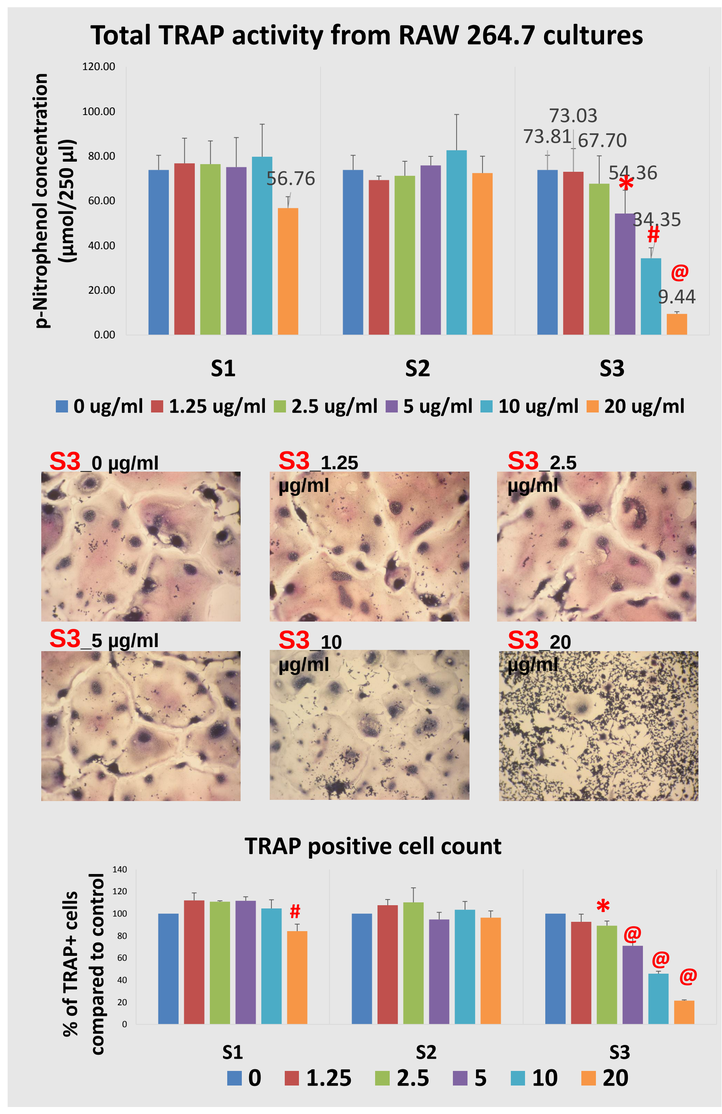


RESULTS: CELL CULTURE

All lipid fractions were cytotoxic at concentrations >32 µg/ml after 1 or 6 days of culture (not shown).

TRAP enzyme activity, which correlates with osteoclast activity, was unaffected by S1 or S2. S3 significantly reduced TRAP in a dose dependant manner (*p<0.05, #p<0.01, @p<0.001 by ANOVA).

Cells stained red, enlarged, and containing >4 nuclei were counted as TRAP-positive osteoclasts. As seen with TRAP enzyme activity, S3 at 2.5 – 20 µg/mL inhibited differentiation of cells into functional osteoclasts; S1 was only effective at 20 µg/mL and S2 had no effect.



CONCLUSIONS

A HFHS diet successfully induced in rats both obesity and an increase in CTX-2, the cartilage degradation marker that is a clinically relevant biomarker of osteoarthritis. The addition of GSM caused a significant reduction in CTX-2, suggesting that consumption of GSM as 30% of daily protein intake and 7% of fat intake can prevent the articular cartilage loss that occurs in osteoarthritis due to obesity and its concomitant chronic inflammation and metabolic syndrome. In vitro, the differentiation of RAW 264.7 pre-osteoclasts when exposed to RANKL into functional bone-resorbing, TRAP-producing osteoclasts was prevented by the addition of non-polar GSM lipids (S3) at concentrations as low as 2.5 µg/mL. The parent fraction (S1) containing all GSM lipids also reduced these effects but only 20 µg/mL. The polar lipid fraction (S2) had no effect. This evidence demonstrates that the non-polar lipids of GSM inhibit osteoclast differentiation in a cell culture model and were likely to be the bioactive component that reduced cartilage damage in the rat obesity model. Further work is ongoing to measure OA in the rat joints as well as biomarkers of chronic inflammation and metabolic syndrome.

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