

## Some significant research outcomes from Honey Research at the University of Waikato

- Establishment of the effectiveness of honey's antibacterial, antifungal and antiviral properties against many microbial species, including antibiotic-resistant strains
- Finding how various types of honeys compare for the potency of their antibacterial and antifungal activity
- Explanation of the variation in level of the special type of antibacterial activity unique to manuka honey
- Proving that methylglyoxal is the component responsible for the non-peroxide antibacterial activity in manuka honey
- Finding that methylglyoxal in manuka honey comes from dihydroxyacetone in the manuka nectar reacting in maturing honey
- Identification of a synergist in manuka honey that doubles the antibacterial activity of methylglyoxal
- Discovering that honey stimulates the immune response of white blood cells
- Finding how various types of honeys compare for the potency of their antioxidant activity
- Discovering pre-emptive antioxidant activity in honey, which stops free radicals from forming
- Demonstrating that honey in the diet is healthier than sugar in respect of health of arteries, immunity, mental deterioration with age, and becoming overweight
- Discovering the way in which the anti-inflammatory activity of honey works
- Discovering how honey works to rapidly remove attached pus and dead tissue from wounds

### These items are shown in more detail below:

(\* indicates collaborative research with the School of Biomedical Sciences, University of Wales Institute Cardiff, UK)

- **Establishment of the effectiveness of honey's antibacterial, antifungal and antiviral properties against many microbial species:**

- **The major wound-infecting species of bacteria (seven species tested)**

Willix, D. J., Molan, P. C. and Harfoot, C. J. (1992) "A comparison of the sensitivity of wound-infecting species of bacteria to the antibacterial activity of manuka honey and other honey." *Journal of Applied Bacteriology* 73 388-394

*Summary:* Both honey and sugar are used with good effect as dressings for wounds and ulcers. The good control of infection is attributed to the high osmolarity, but honey can have additional antibacterial activity because of its content of hydrogen peroxide and unidentified substances from certain floral sources. Manuka honey has been found to have a high level of the latter.

Seven major wound-infecting species of bacteria were studied to compare their sensitivity to the non-peroxide antibacterial activity of manuka honey and to a honey in which the antibacterial activity was primarily

due to hydrogen peroxide. Honeys with activity that was in the middle of the normal range were used. A comparison of the median response of the various species of bacteria showed no significant difference between the two types of activity overall, but marked differences between the two types of activity in the rank order of sensitivity of the seven bacterial species. The non-peroxide antibacterial activity of manuka honey at a honey concentration of 1.8% (v/v) completely inhibited the growth of *Staphylococcus aureus* over an incubation period of 8h. The growth of all seven species was completely inhibited by both types of honey at concentrations below 11% (v/v).

- **Streptococci, bacteria which causes sore throats and wound infections**

Anderson, V. R. (2000) "Investigating the potential for using honey to treat Streptococcal throat infections." MSc Thesis, held in the University of Waikato Library

*Summary:* The aim of this study was to determine if the antibacterial action of honey could be sufficient to be effective against *Streptococcus pyogenes*, the bacterium most commonly responsible for bacterial throat infections, and to determine how honey compares in effectiveness with proprietary antiseptic throat lozenges. The first part of the study was to determine the sensitivity of clinical isolates of *S. pyogenes* to a honey (rewarewa) with an activity due to hydrogen peroxide, to manuka honey which has a unique antibacterial component, and to three proprietary throat lozenges, *Cepacol*, *Strepsils*, and *Dequadin*. The second part of the study was to investigate the effects on growth of bacterial populations of different times of exposure of the bacteria to the antibacterial agents.

Initial research was carried out to determine which clinical isolates of *S. pyogenes* should be used in this study by determining the minimum inhibitory concentrations (MIC) of the manuka honey for nine strains of *S. pyogenes*. These experiments showed that the ML2, ML4 and ML5 strains of *S. pyogenes* had the least sensitivity, middle sensitivity and highest sensitivity respectively to the manuka honey, and they were subsequently used in the rest of the experiments.

The next part of the study was to determine the MIC values for each of the antibacterial agents with the three strains of *S. pyogenes*, without saliva present and with saliva present. These experiments showed that the average MIC for the manuka honey for all three strains was 5.35 ( $\pm$  1.9) % without saliva and 7.04 ( $\pm$  1.2) % with saliva. For the rewarewa honey, it was 8.33 ( $\pm$  2.73) % without saliva and 16.31 ( $\pm$  2.6) % with saliva. For *Dequadin* lozenges it was 15.22 ( $\pm$  2.0) % without saliva and 16.3 ( $\pm$  3.34) % with saliva. For *Strepsils* lozenges it was 11.1 ( $\pm$  1.87) % without saliva and 6.43 ( $\pm$  2.01) % with saliva. For *Cepacol* lozenges the average MIC was 0.41 ( $\pm$  0.10) % without saliva and 1.42 ( $\pm$  0.49) % with saliva. Measurement of the volume of saliva produced when proprietary lozenges or honey lozenges are sucked showed that concentrations of these antibacterial agents around 20% would be achieved in the mouth.

The last part of the study was an investigation of the effect that time of exposure of *S. pyogenes* to the antibacterial agents had on the viability of the bacteria. It was found that only *Cepacol* lozenges had a bactericidal effect on *S. pyogenes* below a concentration of 25% and with less than 60 minutes exposure. The two honeys, at a concentration of 17.5 – 25%, both caused the growth of *S. pyogenes* to be delayed following 5 minutes of exposure, which was similar to the delays in growth caused by the *Dequadin* and *Strepsils* throat lozenges at the same concentrations, in an assay with no saliva present. *Strepsils* lozenges were tested further in an assay with saliva present and it was found that the delays in growth of *S. pyogenes* were substantially increased with saliva. Thus it is concluded that in suppressing the subsequent growth of the bacteria after a short period of exposure *Strepsils* lozenges are more potent than the two honeys with saliva, but the two honeys are of similar potency to *Dequadin*.

Since the rewarewa honey is adversely affected by the presence of saliva, it appears that manuka honey, which is unaffected by saliva, and has an antibacterial activity sufficient to be effective against *S. pyogenes* would be the best to form into a throat lozenge, and is likely to be effective therapeutically.

○ **Staphylococcus aureus, the bacteria which most commonly cause wound infections\***

Cooper, R. A., Molan, P.C. and Harding, K. G. (1999) "Antibacterial activity of honey against strains of *Staphylococcus aureus* from infected wounds." *Journal of the Royal Society of Medicine* 92 283-285

*Summary:* There is rarely any consideration of type of honey used as an antiseptic therapy for infected wounds; nor in *in-vitro* investigations, which have usually been on laboratory-maintained cultures of individual type species, of limited clinical relevance. Furthermore, *in-vitro* investigations have not given any indication of how much variability there may be in the sensitivity of different strains to honey. We tested the sensitivity of 58 strains of coagulase-positive *Staphylococcus aureus*, isolated from infected wounds, to two representative standardised honeys; a pasture honey and a manuka honey. There was a striking similarity between the isolates in their sensitivity to honey: the MIC values (lowest concentration of honey in the agar plates on which there was no sign of growth over 24 h) were all between 2-3% (v/v) for the manuka honey, and between 3-4% (v/v) for the pasture honey. Thus these honeys would still prevent growth of *S. aureus* if diluted by body fluids a further seven- to fourteen-fold beyond the point where their osmolarity ceased to be completely inhibitory.

○ **Coagulase-negative Staphylococci, bacteria which infect catheters passing through the skin\***

French, V. M.; Cooper, R. A. Molan, P. C. (2005) "The antibacterial activity of honey against coagulase-negative staphylococci." *Journal of Antimicrobial Chemotherapy* 56: 228-231

*Summary:* *Objectives:* Development of antibiotic-resistant strains of coagulase-negative staphylococci has complicated the management of infections associated with the use of invasive medical devices, and innovative treatment and prophylactic options are needed. Honey is increasingly being used to treat infected wounds, but little is known about its effectiveness against coagulase-negative staphylococci. The aim of this study was to determine the minimum active dilution of two standardized, representative honeys for 18 clinical isolates of coagulase-negative staphylococci.

*Methods:* An agar incorporation technique was used to determine the minimum active dilution, with dilution steps of 1% (v/v) honey [or steps of 5% (v/v) of a sugar syrup matching the osmotic effect of honey]. The plates were inoculated with 10 mL spots of cultures of the isolates.

*Results:* The honeys were inhibitory at dilutions down to 3.6 – 0.7% (v/v) for the pasture honey, 3.4 – 0.5% (v/v) for the manuka honey and 29.9 – 1.9% (v/v) for the sugar syrup.

*Conclusions:* Typical honeys are about eight times more potent against coagulase-negative staphylococci than if bacterial inhibition were due to their osmolarity alone. Therefore, honey applied to skin at the insertion points of medical devices may have a role in the treatment or prevention of infections by coagulase-negative staphylococci.

○ **Streptococci, bacteria which most commonly cause skin sores and wound infections\***

Cooper, R. A.; Lindsay, E.; Molan, P. C. (2011). "Testing the susceptibility to manuka honey of streptococci isolated from wound swabs." *Journal of ApiProduct and ApiMedical Science* 3 (3), 117-122.

*Summary:* Honey is a broad spectrum antimicrobial agent that has been re-introduced into clinical practice to treat wounds. Wounds support polymicrobial communities of bacteria that either colonise or infect wounds. Strains with resistance to antibiotics are difficult to eradicate and pose a risk of transfer to other patients. Manuka honey has been shown to inhibit many of the bacteria commonly associated with wounds, such as staphylococci, pseudomonads, coliforms and anaerobes, but its efficacy against streptococci isolated from wounds has not been reported. Using macro- and micro-dilution in broth and an agar incorporation technique, the susceptibility to manuka honey of 15 cultures of catalase negative, Gram positive cocci that had been isolated from wounds was tested. All cultures were inhibited by 10% (v/v) manuka honey and statistically significant differences between the three test methods were not found. Manuka honey offers clinical potential in eradicating streptococci from wounds.

○ **MRSA and VRE, antibiotic-resistant "superbugs" which cause wound infections\***

Cooper, R. A.; Halas, E.; Davies, R.; Molan, P. C.; Harding, K. G. (2002) "Honey and gram positive cocci of clinical significance in wounds" *Journal of Applied Microbiology* **93**: 857-863

*Summary:* *Aims:* To determine the sensitivity to honey of gram positive cocci of clinical significance in wounds, and demonstrate that inhibition is not exclusively due to osmotic effects.

*Methods and Results.* 18 strains of methicillin-resistant *Staphylococcus aureus* (MRSA) and 7 strains of vancomycin-sensitive enterococci (VSE) were isolated from infected wounds, and 20 strains of vancomycin-resistant enterococci (VRE) were isolated from hospital environmental surfaces. Using an agar incorporation technique to determine MIC, their sensitivity to two natural honeys of median levels of antibacterial activity was established and compared to an artificial honey solution. For all of the strains tested, MIC values against manuka and pasture honey were below 10% (v/v), but concentrations of artificial honey at least three times higher were required to achieve equivalent inhibition *in vitro*. Comparison of MIC values of antibiotic-sensitive strains with their respective antibiotic-resistant strains demonstrated no marked differences in their susceptibilities to honey.

*Conclusion:* Inhibition of bacteria by honey is not exclusively due to osmolarity. For the gram positive cocci tested, antibiotic-sensitive and resistant strains showed similar sensitivity to honey.

*Significance and Impact of the Study:* A possible role for honey in the treatment of wounds colonised by antibiotic-resistant bacteria is indicated.

**ESBL strains of *E.coli* and other enteropathogens, antibiotic-resistant species which cause gut and wound infections**

Lin, S. M.; Molan, P. C.; Cursons, R. T. (2010) "The controlled *in vitro* susceptibility of gastrointestinal pathogens to the antibacterial effect of manuka honey." *European Journal of Clinical Microbiology & Infectious Diseases* **30** (4), 569-574

*Summary:* The susceptibility of common gastrointestinal bacteria against manuka honey with median level nonperoxide antibacterial activity (equivalent to that of 16.5% phenol) was investigated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a standardized manuka honey with the broth microdilution method. The measured sensitivity of bacteria showed that manuka honey is significantly more effective than artificial honey (a mixture of sugars as in honey), indicating that osmolarity is not the only factor that is responsible for the antibacterial activity of the honey. Most tested gastrointestinal pathogens have MIC and MBC values in the range of 5–10% of honey, other than *Enterobacter* spp. which was in the range of 10–17%. The difference in efficacy between the honey with and without hydrogen peroxide removed was also studied, and it was found that both hydrogen peroxide and the non-peroxide components contribute to the bacteriostatic and bactericidal activity of the honey. It was also found that treatment against multi-antibiotic resistant microorganisms such as *Salmonella typhimurium* DT104 and ESBL-producing organisms with manuka honey may be promising.

○ ***Pseudomonas*, antibiotic-resistant bacteria which infect wounds and burns\***

Cooper, R. A. and Molan, P.C. (1999) "The use of honey as an antiseptic in managing *Pseudomonas* infection." *Journal of Wound Care* **8** (4) 161-164

*Summary:* **OBJECTIVES:** A laboratory study was undertaken to extend existing knowledge about the effectiveness of the antibacterial properties of honey against pseudomonads. To date, sensitivity testing has used non-standardised honeys, which may vary greatly in their antibacterial potency, or laboratory-maintained cultures of individual type species of limited clinical relevance. The present study was undertaken to determine the sensitivity of a broad sample of pseudomonads from infected wounds, to honeys of standardised antibacterial activity.

**STUDY DESIGN:** Pure cultures of *Pseudomonas* spp. isolated from swabs from infected wounds were inoculated on the surface of nutrient agar plates containing various concentrations of honey in the medium.

Two different types of honey were used, a manuka honey and a pasture honey, each selected to have antibacterial activity close to the median for each type.

**STUDY SAMPLE:** The isolates were from 20 different wounds, with chronic and acute infection, of varied aetiology.

**PRIMARY OUTCOME MEASURE:** The minimum concentration of honey in the growth medium needed to completely inhibit the growth of the cultures was determined, within steps of 1% in concentration.

**SECONDARY OUTCOME MEASURE:** The degree of variance between the 20 isolates in their sensitivity to honey was determined, also the relative effectiveness of the two types of honey against the pseudomonads.

**RESULTS:** The minimum inhibitory concentration of the manuka honey for the 20 isolates ranged from 5.5% to 8.7%, with a mean value of 6.9% (v/v) and a standard deviation of 1.3. The minimum inhibitory concentration of the pasture honey for the 20 isolates ranged from 5.8% to 9.0%, with a mean value of 7.1% (v/v) and a standard deviation of 1.0.

**CONCLUSION:** Honey with an average level of antibacterial activity could be expected to be effective in preventing the growth of pseudomonads on the surface of a wound even if the honey were diluted more than ten-fold by exudation from the wound.

- Cooper, R. A.; Halas, E.; Molan, P. C. (2002) "The efficacy of honey in inhibiting strains of *Pseudomonas aeruginosa* from infected burns." *Journal of Burn Care and Rehabilitation* 23 (6): 366-370

**Summary:** Because there is no ideal therapy for burns infected with *Pseudomonas aeruginosa*, there is sufficient need to investigate the efficacy of alternative antipseudomonal interventions. Honey is an ancient wound remedy for which there is modern evidence of efficacy in the treatment of burn wounds, but limited evidence for the effectiveness of its antibacterial activity against *Pseudomonas*. We tested the sensitivity of 17 strains of *P. aeruginosa* isolated from infected burns to two honeys with different types of antibacterial activity, a pasture honey and a manuka honey both with median levels of activity. All strains showed similar sensitivity to honey with Minimum Inhibitory Concentrations (MIC) below 10%(v/v); both honeys maintained bactericidal activity when diluted more than ten-fold.

Honey with proven antibacterial activity has the potential to be an effective treatment option for burns infected or at risk of infection with *P. aeruginosa*.

- **Fungal species that cause tinea such as athlete's foot and ringworm**

Brady, N.F., Molan, P.C. and Harfoot, C.G. (1997) "The sensitivity of dermatophytes to the antimicrobial activity of manuka honey and other honey." *Pharmaceutical Sciences* 2, 1-3

**Summary:** Honey has been reported to have antifungal activity, so was tested against clinical isolates of the common dermatophyte species which cause tinea in humans. A honey with an average level of hydrogen peroxide, and a manuka (*Leptospermum scoparium* J.R and G. Forst.: Family Myrtaceae) honey with an average level of non-peroxide antibacterial activity were used. An agar well diffusion assay was used, the contents of the wells being replaced with freshly prepared honey solutions at 24 hour intervals over the 3 - 4 days of incubation.

The lowest concentrations (% v/v, in steps of 5%) of manuka honey with catalase added to remove hydrogen peroxide, and of the other honey (without catalase added) that showed inhibition of growth around the wells were, respectively: with *Epidermophyton floccosum* 25%, 10%; *Microsporum canis* 25%, 15%; *M. gypseum* 55%, 20%; *Trichophyton mentagrophytes* var. *interdigitale* 45%, 15%; *T. mentagrophytes* var. *mentagrophytes* 25%,15%; *T. rubrum* 20%, 5%; *T. tonsurans* 25%, 20%. No inhibitory activity was detected with the other honey at 50% (v/v) with catalase added.

The results of this investigation thus show that the common dermatophytes are fairly sensitive to the antimicrobial activity of honey, indicating that clinical evaluation of honey in the treatment of tinea is

warranted. This would determine whether the hydrogen peroxide or the non-peroxide antifungal agent diffuses better into the skin.

○ **Gastroenteritis-causing bacteria**

Lin, S. M.; Molan, P. C.; Cursons, R. T.(2008) "The in vitro susceptibility of *Campylobacter* spp. to the antibacterial effect of manuka honey." *European Journal of Clinical microbiology and Infectious Diseases* **28**: 339-344

*Summary:* We report the antimicrobial effect of manuka honey against *Campylobacter* spp. isolated by a diagnostic laboratory from specimens from a community in New Zealand. The isolates were differentiated according to species level using multiplex PCR. *C. jejuni* (20 strains) and *C. coli* (7 strains) were identified. The clinical isolates identified and type culture collection strains of these species were subjected to testing to determine the minimum inhibitory concentration (MIC) of manuka honey using a microdilution technique. The MIC of the manuka honey against all of the *Campylobacter* tested was found to be around 1% (v/v) honey. The low MIC values suggest that honey might still inhibit the growth of campylobacteria after dilution by fluid in the gut, but the actual concentration of honey that can be achieved in the intestine is unknown. Therefore, clinical investigation is required to establish the efficacy of honey against *Campylobacter* spp. in the gut environment.

Lin, S.-M. (2010) "The effect of manuka honey on Enterobacteria." PhD Thesis, held in the University of Waikato Library (Downloadable pdf available: <http://researchcommons.waikato.ac.nz/handle/10289/3972> )

*Summary:* Manuka honey (*Leptospermum scoparium*) produced in New Zealand has been shown to exhibit substantial antibacterial activity against a broad range of pathogens causing wound infection, and is being used in wound management with excellent results. This activity is due to both hydrogen peroxide and non-peroxide components. Manuka honey, however, may not be useful for treating bacterial gastroenteritis because the gastrointestinal environment may be unfavourable to the antibacterial action, and because a sufficiently high concentration for effectiveness may not be achieved. The research in this thesis is set out to evaluate in vitro the efficacy of manuka honey as an antibacterial agent against enterobacteria, taking into consideration some factors that may be involved in the gastrointestinal environment.

Because some gastrointestinal bacteria (*Campylobacter* spp., *Helicobacter pylori*, *Lactobacillus* spp. and *Bifidobacterium animalis* subsp. *lactis*) are not aerophilic, a cheap yet acceptable gas generating system alternative to the commercial gaspack counterpart was sought for use in this study. Various alternatives were compared for their performance. The spirits burn method was chosen for cultivating microaerobes and some anaerobes because of its comparable performance to that of commercial systems in terms of the growth of bacterial species, and because of the ease of use and the low cost.

In the first part of this thesis, the susceptibility of gastrointestinal bacteria against manuka honey was investigated by determining the minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) using a standardised manuka honey. Throughout the research, a manuka honey with median level non-peroxide antibacterial activity (equivalent to that of 16.5% phenol) was used, except that *Campylobacter* spp. were assayed with a more potent manuka honey equivalent to 29.4% phenol. The measured sensitivity of bacteria showed that manuka honey is significantly more effective than artificial honey (a mixture sugars as in honey), indicating that osmolarity is not the only factor that is responsible for the antibacterial activity of the honey. It was found that some species of bacteria e. g. *Campylobacter* spp. are exceptionally sensitive to manuka honey (both MIC and MBC are about 1% honey solution), whereas most other gastrointestinal pathogens have MIC and MBC values in the range 5–10% honey other than *Enterobacter* and *Pseudomonas* which were in the range 10–17%. *Bifidobacterium*, *Lactobacilli* and *enterococci* appear to be more tolerant to the honey (MIC: 9.36–14.29%; MBC:  $\geq$ 13.3%) than most other species are. The difference in efficacy between the honey with and without hydrogen peroxide removed was also studied, and it was found

that both hydrogen peroxide and the non-peroxide components contribute to the bacteriostatic and bactericidal activity of the honey.

Because oxygen is required for hydrogen peroxide to be produced in honey, the role that oxygen plays in the antibacterial activity of manuka honey was investigated by analysing the susceptibility data obtained under both aerobic and anaerobic conditions using facultative anaerobes. Manuka honey appeared to be a more potent bacteriostatic agent against most species of bacteria in the absence of oxygen, whereas a relatively higher concentration of manuka honey solution was required to kill some bacteria under anaerobic conditions. This may partially be due to the atmosphere having also affected the metabolism, and hence the growth, of bacteria. Therefore, the activity of manuka honey would not necessarily decline in the intestinal environmental atmosphere.

To investigate how long it takes for manuka honey to kill bacteria, time-to-kill studies were conducted by monitoring the survival of bacteria in manuka honey. It is found that it takes a 20% solution of manuka honey with a medium-level activity more than 6 h to kill 90% of the cells of most of the species tested if the bacterial cells are kept in contact with the honey. This suggests that manuka honey is not rapidly bactericidal, and that it is unlikely to be possible to fully eradicate a bacterial gut infection by ingesting a small amount of manuka honey for a short period. It was found that probiotics can survive in the 20% honey solution for more than 12 h.

The pharmacodynamics of the antibacterial activity of manuka honey were studied to investigate the survival and the re-growth of bacteria after they had been treated with honey. It was revealed that after being exposed to manuka honey for a short term (1 h), the growth of most enteropathogens is slowed for approximately 2–4 h before it gets back to a full rate. The assays of this postantibiotic effect also showed that the latency in the re-growth after being exposed to honey is not proportional to the MIC, MBC or time-to-kill profiles.

Finally, the efficacy of manuka honey on bacteria was studied under conditions simulating the environment in the stomach and intestines. The tested bacteria were unable to grow under the acidic conditions as in the stomach, so whether or not the honey had any antibacterial activity under these conditions could not be determined. Under the conditions simulating the intestinal environment, the results demonstrated that the antibacterial activity of manuka honey is slightly decreased in the mildly alkaline conditions of the intestine (pH 7.5). In the presence of pancreatin and bile at the same pH, the activity of manuka honey was found to decrease by more than 50%. This suggests that pancreatin and bile in the gut may negatively affect the efficacy of the antibacterial activity of manuka honey *in vivo*. This indicates that although ingested manuka honey may still have some antibacterial action when in the gut, the antibacterial activity would be different from that which is usually examined with sensitivity studies *in vitro*.

Gastroenteritis has generally been treated with oral rehydration solution (ORS) that consists of carbohydrates and electrolytes. Manuka honey could be used instead of the usual carbohydrate component of ORS and would provide additional bioactivities such as antibacterial activity and stimulation of growth of probiotics, which would make the honey rehydration solution more beneficial to patients with gastroenteritis than is the traditional ORS. After some initial investigation to find the most appropriate dosage and frequency of doses, a clinical trial may be warranted.

Ko, Y.-J. (2005) "Investigating the sensitivity of enteropathogenic bacteria to the antibacterial activity of honey ." MSc Thesis, held in the University of Waikato Library

**Summary:** Gastroenteritis is a common syndrome occurring world wide, causing more than five million deaths each year. Infection with enteropathogenic bacteria is one of the major causes of this disease and is commonly treated with antibiotics. The toxicity and side-effects of antibiotics and the bacterial resistant caused by it has restricted the use of it in treating bacterial infections. Honey has been used as medicine in ancient history and folk medicine, treating various types of infections. The rediscovery of its therapeutic property and the success of it in treating wound infection have indicated the potential of the use of honey in treating

bacterial gastroenteritis. The aim of this study was to investigate the sensitivity of enteropathogenic bacteria to the antibacterial activity of honey.

Clinically isolated bacteria were used in this study, including species of *Aeromonas*, *Escherichia coli*, *Salmonella*, *Shigella*, *Yersinia* and *Campylobacter*. The isolates being tested to find their sensitivity to honey, a number of isolates (between five to twenty) were tested for each group of bacteria to evaluate the variability of sensitivity to honey within a group. Two standardized honeys with a similar level of antibacterial activity were tested, a manuka honey with antibacterial activity due mainly to an unidentified phytochemical component, and a pasture honey with antibacterial activity due to hydrogen peroxide alone.

A sensitivity assay was used to investigate the sensitivity of these bacteria to the two honeys. Standardised bacterial cultures were dispensed into different concentrations of honey in broth in the wells of a microtiter plate then incubated for eighteen hours in a microplate reader at the optimum growing temperature for the bacteria. The lowest concentration of honey for which there was no growth was taken as the minimum inhibitory concentration (MIC) for the isolate tested.

All the isolates tested were sensitive to the two honeys tested, except for the case of *Campylobacter*, where no results were obtained because of difficulties getting it to grow. The MIC of the manuka honey (with antibacterial activity equivalent to 23.4% phenol) ranged from 3.5% to 5.8% (v/v) honey and the MIC of the pasture honey (with antibacterial activity equivalent to 26.1% phenol) ranged from 4.9% to 8.6% (v/v) honey. The low MIC values indicate that the antibacterial activity of honey could be feasibly obtained in the gut even after heavy dilution by digestive fluids. Therefore, it is concluded that honey may be an effective treatment for bacterial gastroenteritis, and clinical trials are warranted.

○ ***Helicobacter pylori*, a bacterium which causes stomach ulcers**

Al Somai, N., Coley, K.E., Molan, P.C. and Hancock, B.M. (1994) "Susceptibility of *Helicobacter pylori* to the antibacterial activity of manuka honey." *Journal of the Royal Society of Medicine* **87** (1) 9-12

*Summary:* Honey is a traditional remedy for dyspepsia, and is still used for this by some medical practitioners although there is no rational basis for its use. The finding that *Helicobacter pylori* is probably the causative agent in many cases of dyspepsia has raised the possibility that the therapeutic action of honey may be due to its antibacterial properties. Consequently, the sensitivity of *Helicobacter pylori* to honey was tested, using isolates from biopsies of gastric ulcers. It was found that all five isolates tested were sensitive to a 20% (v/v) solution of manuka honey in an agar well diffusion assay, but none showed sensitivity to a 40% solution of a honey in which the antibacterial activity was due primarily to its content of hydrogen peroxide. Assessment of the minimum inhibitory concentration by inclusion of manuka honey in the agar showed that all seven isolates tested had visible growth over the incubation period of 72 h prevented completely by the presence of 5% (v/v) honey.

○ **Bacteria causing mastitis in cattle**

Allen, K.L. and Molan, P.C. (1997) "The sensitivity of mastitis-causing bacteria to the antibacterial activity of honey." *New Zealand Journal of Agricultural Research* **40**., 537-540

*Summary:* The use of honey as a wound dressing is well established in traditional and modern medicine. There are many reports of its effectiveness in clearing bacterial infections in ulcers and abscesses, which suggest that it may be suitable for the intramammary treatment of mastitis. To evaluate this possibility, the species of bacteria that commonly cause mastitis in dairy cows were tested for their sensitivity to the antibacterial activity of honey. The growth of all seven species tested was completely inhibited by a typical honey (with antibacterial activity due to its content of hydrogen peroxide) at a concentration of 10% (v/v) in the agar plates, and two by 5% honey. Six of the species were completely inhibited by a typical manuka honey (with antibacterial activity due to its content of a non-peroxide component) at a concentration of 5% (v/v). Only one species was inhibited by 10% (v/v) artificial honey (sugars and gluconic acid as in honey). As honey is



harmless to tissues and would leave no undesirable residues in milk it would be of interest to now evaluate it therapeutically in clinical mastitis.

○ **Bacteria causing periodontitis (infection of the gums)**

Svenson, E. L. (2004) "Investigating the potential of using honey for therapy of periodontal disease." MSc Thesis, held in the University of Waikato Library

*Summary:* For as long as written records have existed honey has been used as a medicine for the treatment of wounds and infections. The knowledge of the different types of microorganisms susceptible to the antimicrobial properties of honey is expanding. The purpose of this thesis is to study the antimicrobial effects of Manuka, pasture and artificial honey on a range of anaerobic periodontal pathogens, including bacteria and yeasts. Previous studies have looked at the effect of modern antibiotics on these pathogens, with a small number of studies investigating the effect of Propolis and plant extracts on the pathogens.

This study investigated 12 species of common anaerobic periodontal bacteria (*Actinobacillus actinomycetemcomitans*, *Actinomyces gerencseriae*, *Actinomyces nueslundii*, *Actinomyces odontolyticus*, *Campylobacter rectus*, *Eikenella corrodins*, *Fusobacterium nucleatum*, *Peptostreptococcus micros*, *Porphyromonas gingivalis*, *Prevotella buccae*, *Prevotella intermedia* and *Veillonella parvula*) as well as 3 species of periodontal yeasts (*Candida albicans*, *Candida glabrata* and *Pichia guilliermondii*). It was found that all species of bacteria (except *Actinomyces odontolyticus*, *Campylobacter rectus*, and *Pichia guilliermondii* as they were unable to be cultured) were susceptible to the antimicrobial properties of honey. For bacteria, it was found that minimum inhibitory concentrations of Manuka honey (with a non-peroxide activity equivalent to 15% (w/v) phenol) ranged between 5.11% and 9.1% (v/v) honey. For Yeasts the minimum inhibitory concentrations of Manuka honey ranged between 15.8% for *Candida albicans* and 40% for *Candida glabrata*. Conversely the minimum inhibitory concentrations of pasture honey (with a peroxide activity equivalent to 15% (w/v) phenol) ranged between 4% and 9.8% (v/v) honey for bacteria and for yeasts, the minimum inhibitory concentration were 40% for both *Candida albicans* and *Candida glabrata*. It can be concluded that Manuka honey and pasture honey are likely to be effective antimicrobial agents in the treatment of bacteria that cause periodontitis, while Manuka and pasture honey are not likely to be effective in the treatment of yeasts in periodontitis.

The second part of the study investigated the length of exposure to Manuka honey required to show a bactericidal effect or to inhibit the growth of selected bacteria. The time-to-kill results obtained show a reduced survival rate ranging between 25 minutes and 8 hours. Considerably reduced survival was observed at the 8 hour treatment period with many assays showing complete death of the culture.

The last part of the study was to investigate whether the antimicrobial properties of Manuka honey could diffuse through the oral tissue to the site of infection. The preliminary investigation of the diffusion of honey through oral tissue was inconclusive with regard to the penetration of the antimicrobial components of Manuka honey through the oral tissue. Additional tests should be carried out on this topic to develop a reliable method.

○ **Adenovirus (which causes respiratory infections and eye infections) and herpes simplex virus (which causes cold sores and genital sores)**

Littlejohn, E. S. V. (2009) "The sensitivity of Adenovirus and Herpes simplex virus to honey." MSc Thesis, held in the University of Waikato Library

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*Summary:* Honey has been used for centuries as a medicine to treat various ailments and infections. A large amount of research has established that honey has potent antibacterial activity. However, the sensitivity to honey of viral species that cause infections has been studied in only a small number of cases. The aim of this study was to obtain data to clarify and extend knowledge obtained from these previous studies of honey's

antiviral activity, and especially study those viruses that cause localised infections which have limited or no therapy available, which are suitable to treatment with topically applied honey.

The susceptible A549 cell line and viral isolates of Adenovirus serotypes 1, 3, and 8, and Herpes simplex virus serotypes 1 and 2, were provided by the Waikato Hospital Virology Laboratory. A number of types of honey were investigated from a range of sources: Manuka honey with high concentrations of methylglyoxal, unique manuka factor activity, and phenolics, Honeydew and Rewarewa honeys which have high antioxidant activity, and Ling Heather honey which is high in phenolic compounds. These honeys were selected due to their range of characteristic activities in order to make comparisons with antiviral activity.

A variety of tests using cell culture were developed to evaluate the sensitivity of the viruses to whole honey. Each test scored and monitored the development of morphological changes to the cells, to observe whether the honey treatment can prevent the development of these changes known as viral cytopathic effect. These included tests for: protection, in which the cells were pre-treated with, and incubated either with or without honey; prevention, where honey was used to treat infected cells, and in plaque reduction assays, to examine whether it can reduce the resultant number of plaques; and neutralisation, in which the virus was directly exposed to the honey for a defined period.

It was found with each type of test using cell culture that many of the honeys studied can lower the severity of viral cytopathic effect or delay its onset compared with the development observed with virus that was not treated with honey. This can suggest that the antiviral activity may be a feature of more than one type of honey. In general the antiviral effect increased with the concentration of honey and time the virus was exposed to it. Manuka honey M116 at a concentration of 10% was effective in preventing the development of viral cytopathic effect of each of virus, after the viruses at concentrations in excess of the tissue culture infectious dose had been exposed to the honey for 8 hours.

Enzyme-linked immunosorbant assays were used to measure the effect the successful treatments found in the extended neutralisation experiments had on viral surface proteins necessary for viral entry into the cells. The results using this technique suggested that there was very little virus present in the samples that had been treated with honey and with the untreated virus. Therefore it could not be shown whether the honey was acting via this mechanism. It is concluded from the findings in this study that honey is likely to be an effective antiviral treatment for the therapy of localised viral infections, this needs to be verified by clinical trials.

○ **Respiratory Syncytial Virus (which is the major cause of viral respiratory infections in children)**

Zareie, P. P. (2011) "Honey as an antiviral agent against respiratory syncytial virus" MSc Thesis, held in the University of Waikato Library

(Downloadable pdf available: <http://researchcommons.waikato.ac.nz/handle/10289/5291> )

*Summary:* Respiratory syncytial virus is the most frequent cause of hospitalization for viral respiratory infections in infants and young children worldwide. It also severely affects immunocompromised adults and the elderly, however, despite decades of efforts, there is no proven effective treatment for RSV infection and attempts at vaccine development have been hampered by several major obstacles. A large amount of research has established the potent antibacterial activity of honey, but its activity against viral species has been the subject of only a small number of studies. These were with viruses which cause localised infections in which honey could be used topically. Recent studies demonstrating the safety of intrapulmonary administration of honey in sheep and humans raised the possibility of using honey to treat respiratory infections. The aim of this study, therefore, was to extend the knowledge obtained from previous studies of honey's antiviral activity to its action against RSV. A variety of tests using cell culture were developed to evaluate the susceptibility of RSV to honey. Each test monitored and scored the development of morphological changes to the cells caused by RSV infection to determine whether the honey had any inhibitory effect on these changes. These included tests for: inhibition, where honey was used to treat infected cells; protection, in which the cells were treated with honey prior to infection; neutralisation, in which the virus was directly exposed to the honey for a defined period before being used to inoculate the cells. Pre-treatment of the cells had no effect on the consequent

development of cytopathic effect, while the inhibition and neutralisation experiments showed a significant inhibitory effect on the progression of infection, suggesting a direct effect on the virus rather than on the cells, however, further studies are required to confirm this. A wide range of honey types were tested for their inhibitory and neutralising capabilities against RSV and the results suggested that the antiviral activity may be characteristic of more than one type of honey. The activity observed did vary, however, with some types of honey causing greater inhibition of RSV than others. Enzyme-linked immunosorbent assays were also used to quantitatively measure the number of viral antigens in honey-treated and untreated cells. The results confirmed that treatment with honey had caused inhibition of viral replication, there being very little virus detected in honey-treated cells compared with untreated cells infected with RSV. Experiments using quantitative PCR also demonstrated the inhibitory effect of honey on RSV at the transcription level, with significant differences in the mRNA copy numbers of two out of the three viral genes examined. Attempts at isolating the antiviral component in honey demonstrated that the sugar was not responsible for the inhibition of RSV, but that methylglyoxal may play a part in the greater potency of Manuka honeys against RSV. It is concluded from the findings in this study that honey may possibly be an effective antiviral treatment for the therapy of respiratory viral infections, and provides justification for future in vitro studies and clinical trials.

- **We have established through surveys of New Zealand honey varieties how they compare for antibacterial and antifungal activity**

Allen, K.L., Molan, P.C. and Reid, G.M. (1991) "A survey of the antibacterial activity of some New Zealand honeys." *Journal of Pharmacy and Pharmacology* **43** (12) 817-822

**Summary:** There is increasing usage of honey as a dressing on infected wounds, burns and ulcers, yet no regard is given to which type of honey is used. A survey was carried out to assess the variation in antibacterial activity of honey, 345 samples of unpasteurised honey being obtained from commercial apiarists throughout New Zealand. Most of these honeys were considered to be monofloral, from 26 different floral sources. The honeys were tested against *Staphylococcus aureus* in an agar well diffusion assay, with reference to phenol as a standard. Antibacterial activity was found to range from the equivalent of <2%(w/v) phenol to 58%(w/v) phenol, with a median of 13.6 and a standard deviation of 12.5.

Neither the age of the honey samples (1 month to 6 years old) nor whether they had been processed by the apiarist or not was associated with lower activity.

The difference between floral sources in the antibacterial activity was very highly significant. However, because there is so much variance within floral types it is necessary for honey that is to be used as an antiseptic to be assayed for its antibacterial activity first. Kanuka, manuka, ling heather and kamahi showed to be sources likely to give a honey with high antibacterial activity.

When antibacterial activity was assayed with catalase added to remove hydrogen peroxide, most of the honeys showed no detectable antibacterial activity. Only manuka and vipers bugloss honeys showed this type of activity in a significant proportion of the samples. The fairly high antibacterial activity of manuka honey was in many cases due entirely to this non-peroxide component.

Brady, N., Molan, P., Bang, L. (2004) A survey of non-Manuka New Zealand honeys for antibacterial and antifungal activities. *Journal of Apicultural Research* **43** (2): 47-52

**Summary:** To assess the variation in antibacterial and antifungal activity of non-manuka honeys, a study was undertaken using 179 unifloral, unpasteurized honey samples obtained from commercial beekeepers throughout New Zealand. The honeys were tested against *Staphylococcus aureus* and *Candida albicans*, *Escherichia coli* and the dermatophyte *Trichophyton mentagrophytes* using agar well diffusion, measurement of minimum inhibitory concentration and modified agar well diffusion methods, respectively. Of the 179 non-manuka honey samples

assessed, none showed non-peroxide or anti-yeast antimicrobial activities. By contrast, 50% of the samples tested showed antibacterial activity against *S. aureus*, with activity ranging from 5.0–27.9% phenol equivalent. Approximately 30% of the samples tested showed antibacterial activity against *E. coli*; however honey concentrations required for inhibition were, with one exception, in excess of 19%. Similarly, 35% of samples showed antifungal activity although the levels of activity measured were low.

- **We have found the explanation for the level of non-peroxide antibacterial activity in manuka honey varying widely**

Stephens, J. McD. C. (2006) "The factors responsible for the varying levels of UMF<sup>®</sup> in manuka (*Leptospermum scoparium*) honey." PhD Thesis, held in the University of Waikato Library (Downloadable pdf available: <http://researchcommons.waikato.ac.nz/handle/10289/2655>)

*Summary:* The variability in the level of the non-peroxide antibacterial component (UMF<sup>®</sup>) of manuka honey produced in New Zealand was studied. A field analysis confirmed considerable variability existed in the honeys, and a number of hypotheses to explain this variability were proposed and examined.

Nectar derived from *Leptospermum scoparium* (manuka), was confirmed to be the source of UMF<sup>®</sup>.

The dilution of manuka honey with nectar derived from other floral sources was found to proportionally reduce the UMF<sup>®</sup> in monofloral manuka honey. The utilisation of the thixotropic properties of manuka honey allowed the degree of dilution in the field samples to be established, and an adjustment of the field results to account for the dilution of UMF<sup>®</sup> by other honey types revealed all monofloral manuka honey contains UMF<sup>®</sup>. However, in the monofloral manuka honey, significantly different levels of UMF<sup>®</sup> activity were found to come from reasonably well-defined geographic regions.

The cause of the variable levels of UMF<sup>®</sup> activity in manuka honey would appear to be the different varieties of *L. scoparium* being harvested by the honeybees, and the environmental parameters influencing nectar production or another species interacting with *L. scoparium* do not appear to influence UMF<sup>®</sup> activity.

Three methods were used to establish genetic variability within regions of the North Island of New Zealand that gave rise to the various levels of UMF<sup>®</sup> activity. Analyses of morphological characteristics, chemotaxonomic essential oil profiles, and population genetics of *L. scoparium* populations were conducted, and the conclusions that were drawn from each of these were very similar. Four varieties were identified, divided into two divisions. The northern division, which contained the core populations from Northland and Waikato, represented the previously described *L. scoparium* var. *incanum* and *L. scoparium* var. *linifolium*. This division yielded manuka honey with high UMF<sup>®</sup> activity. The southern division, which contained the core populations from the Central North Island and East Coast, represented the previously described *L. scoparium* var. *myrtifolium* and an unnamed variety. The latter, growing principally on the East Coast, uniquely contains triketones essential oils. The southern division yielded manuka honey with low UMF<sup>®</sup> activity. Hybridisation between these varieties will occur, leading to a continuum of UMF<sup>®</sup> activity in manuka honey.

The data indicated multiple dispersions of *L. scoparium* to New Zealand from the evolutionary centre of the persistent-capsule *Leptospermum* group in south-east Australia, and later regional dispersal in New Zealand.

From this study two hypotheses were accepted: the variability in the UMF<sup>®</sup> activity of manuka honey is due to both the dilution of manuka honey by other honey types and the variety of *L. scoparium* harvested.

- **We have proven by quantitative measurement that the non-peroxide antibacterial activity of manuka honey is due to methylglyoxal**

Adams, C. J.; Boulton, C. H.; Deadman, B. J.; Farr, J. M.; Grainger, M. N.; Manley-Harris, M., and Snow, M. J. (2008). Isolation by HPLC and characterisation of the bioactive fraction of New Zealand manuka (*Leptospermum scoparium*) honey. *Carbohydrate Research* 343(4), 651-659

*Summary:* Using HPLC a fraction of New Zealand manuka honey has been isolated, which gives rise to the non-peroxide antibacterial activity. This fraction proved to be methylglyoxal, a highly reactive precursor in the formation of advanced glycation endproducts (AGEs). Methylglyoxal concentrations in 49 manuka and 34 non-manuka honey samples were determined using a direct detection method and compared with values obtained using standard o-phenylenediamine derivatisation. Concentrations obtained using both the methods were similar and varied from 38 to 828 mg/kg.

- **We have found that methylglyoxal in manuka honey forms in a reaction in the honey of dihydroxyacetone from the manuka nectar**

Adams, C.J., Manley-Harris, M., Molan, P.C. (2009) "The origin of methylglyoxal in New Zealand manuka (*Leptospermum scoparium*) honey." *Carbohydrate Research* 344: 1050-1053

*Summary:* Methylglyoxal in New Zealand manuka honey has been shown to originate from dihydroxyacetone, which is present in the nectar of manuka flowers in varying amounts. Manuka honey, which was freshly produced by bees, contained low levels of methylglyoxal and high levels of dihydroxyacetone. Storage of these honeys at 37 degrees C led to a decrease in the dihydroxyacetone content and a related increase in methylglyoxal. Addition of dihydroxyacetone to clover honey followed by incubation resulted in methylglyoxal levels similar to those found in manuka honey. Nectar washed from manuka flowers contained high levels of dihydroxyacetone and no detectable methylglyoxal.

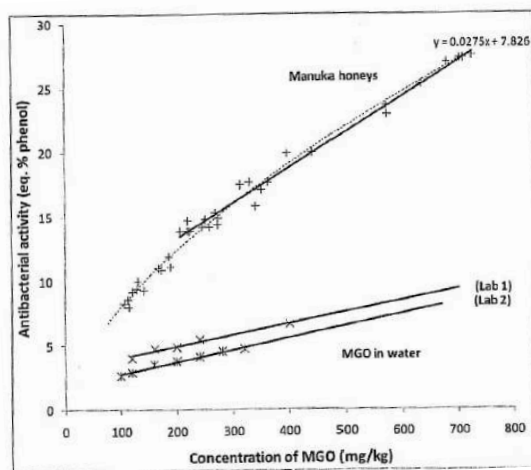
- **We have found that there is a synergist in manuka honey which doubles the antibacterial activity of methylglyoxal**

A paper is being produced for publication in a journal. A magazine article outlining this work has been published: Molan, P. C. (2008) "An explanation of why the level of MGO in manuka honey does not show the antibacterial activity." *New Zealand Beekeeper* 16 (5) 11-13

*Summary:* The antibacterial activity of manuka honey is due to synergy between methylglyoxal (MGO) and non-antibacterial components in the honey. This synergy accounts for half or more of the non-peroxide antibacterial activity of manuka honey.

The antibacterial activity of MGO is far less when it is in water than when it is in honey – it has less than half of the antibacterial activity that is seen when the same level is in manuka honey. This is scientific proof that the MGO present does not by itself account for the non-peroxide antibacterial activity of manuka honey.

Increased levels of MGO just add to the base level of activity, which is why the antibacterial activity of the honey does not increase in proportion to the level of MGO.



Note: The data that was published in this graph for the samples of manuka honey was taken from Adams *et al.* (2008) *Carbohydrate Research* 344: 1050-3. Adams *et al.* subsequently published in *Carbohydrate Research* 344: 2609 that they had made an error in calculation, and that the values for the content of MGO in each sample should each have been 87% higher. That made the results out of line with two other published sets of data. For a discussion of this see:

<http://www.academia.edu/attachments/3023960>

- **We have established that honey has a stimulatory effect on the immune response of white blood cells\***

Tonks, A.; Cooper, R. A.; Price, A. J.; Molan, P. C.; Jones, K. P. (2001) "Stimulation of TNF- $\alpha$  release in monocytes by honey." *Cytokine* 14 (4) 240-242

**Summary:** Although evidence exists for the antibacterial effects of honey there is limited objective evidence for direct promotion of healing. We investigated the effect of manuka, pasture and an artificial honey on macrophage function. Reactive oxygen intermediate (ROI) production was assessed by luminol enhanced chemoluminescence and tumour necrosis factor-alpha (TNF- $\alpha$ ) release was determined by immunoassay. ROI production was significantly ( $P < 0.001$ ) decreased by pasture honey and manuka honey. TNF- $\alpha$  release was significantly enhanced ( $P < 0.001$ ) in unprimed MM6 cells by manuka and pasture honey but was not altered in primed cells. These results could explain the suggested therapeutic properties of honey in promoting wound healing.

Harcourt, N. R. (2005) "The effects of honey on the inflammatory response of cells with respect to wound healing" PhD Thesis, held in the University of Waikato Library

**Summary:** The primary aim of this thesis was to test the effect of two types of honey, a manuka honey and an Otago pasture honey, on a range of key cell types involved in the inflammatory response of wound healing. A range of *in vitro* assays were used to test the effect of honey on various cells with a view to the implications of the results for wound healing, focusing on the mechanisms by which honey has been observed to have beneficial effects on both scar formation and inflammation.

To investigate whether honey could stimulate bovine T cells to proliferate *in vitro*, both MTT and BrDU assays of proliferation and flow cytometry analysis were used. It was found that low concentrations of honey stimulated resting T cells to proliferate and express the IL-2 receptor in a dose-dependent manner with progressive dilution. This suggests that honey contains lymphomitogenic factors. Manuka honey was stimulatory at higher dilutions than pasture honey. Low concentrations of honey induced cell division profiles similar to those obtained with Con A-stimulated cells. The stimulatory activity of honey was found to be in a high molecular weight fraction. Sugars alone had no effect on T cell proliferation, as demonstrated by use of artificial honey (a syrup of sugars as in honey).

The ability for honey to induce messenger RNA expression for key cytokines involved in wound healing was investigated. Conventional reverse transcriptase-PCR was used to detect the production of mRNA for honey at 0.25% concentrations for various times (0-24 h). The more sensitive molecular technique, quantitative real-time RT-PCR was then used to quantify the abundance of cytokine mRNA transcripts expressed in bovine blood

exposed to 0.25% manuka honey as compared with Con A or control cultures. Transcriptional activity of ten genes, IL-1, IL-5, IL-12, IL-18, IFN- $\gamma$ , HSP70, HSP90, i-NOS, TNF- $\alpha$ , and TGF- $\beta$  were studied at the mRNA level during a 0–24 h exposure of whole blood to honey. To test for any modulatory effects of honey on gene expression in an inflammatory model, whole blood was exposed to honey at the same time as LPS and the mRNA expression for the genes was measured. The results show that honey up-regulates a wide range of mediators, including TNF- $\alpha$ , IL-1 $\beta$ , and TGF- $\beta$ , and this supports the hypothesis that honey induces cytokine release. Honey gave a transient and moderate induction of cytokine mRNA compared with a massive and prolonged induction by the mitogens, Con A and LPS. The inclusion of honey with LPS led to a reduced expression of mRNA for key inflammatory mediators, including iNOS and TGF- $\beta$ , compared with LPS alone. This supports the hypothesis that honey modulates inflammation.

To investigate whether honey could induce THP-1 monocytes to release TNF- $\alpha$ , bioassays using WEHI cells were carried out to measure TNF- $\alpha$  production after the monocytes were exposed to honey. Honey was found to stimulate release of TNF- $\alpha$  by the monocytes when at a range of concentrations between  $2.5 \times 10^{-5}$ – $1 \times 10^{-1}$ , with no differences between the levels produced at the various concentrations of honey. At concentrations of honey from 0.25–1% the TNF- $\alpha$  production decreased as the concentration of honey increased. This may indicate that an anti-inflammatory action overrides the stimulatory effect at concentrations of honey greater than 0.25%. Sugar content had no effect upon TNF- $\alpha$  release, as demonstrated by the artificial honey control. There were no differences between honey types (manuka honey and pasture honey) in induction of TNF- $\alpha$  release. Time-course analysis confirmed that a 4–6 h incubation period of cells with 0.25% honey gave maximal TNF- $\alpha$  production. A 2 h minimum exposure period of cells to honey was critical for TNF- $\alpha$  production. Incubation of LPS-stimulated monocytes with honey had no effect on their subsequent TNF- $\alpha$  production. A good correlation was found between the TNF measurements detected by ELISA and the WEHI Bioassay.

To test whether honey could modulate LPS-stimulated NO production by THP-1 monocytes and bovine peripheral blood mononuclear cells, Greiss assays were performed. Both manuka honey and pasture honey at 0.5% and 1% concentrations suppressed LPS-induced nitrite release in a dose-wise manner, indicating modulation of nitric oxide production. Manuka honey had a more potent modulating effect on LPS-driven nitrite production than pasture honey, and maintained activity at 0.25%. Sugars alone had no effect. High molecular weight dialysis fractions of either honey contained the activity, but some of the activity was lost by fractionation. An ether extract of manuka honey led to the greatest modulation of nitrite production by LPS-stimulated monocytes.

To investigate whether honey has an effect on phagocytosis, whole blood was incubated with honey and the ability of neutrophils to take up fluorescent-labelled bacteria was measured using the Phagotest<sup>®</sup> assay. The artificial honey control provided clear evidence that low concentrations of honey (optimal at 0.25%) induce phagocytosis by neutrophils due to the supply of sugars. Manuka honey had an additional opsonizing effect on bacteria, which enhances the phagocytic response beyond that seen with sugars alone.

The effects of honey on tight junction (TJ) resistance were assessed for MDCK cell monolayers subjected to an EGTA challenge. It was found that manuka honey and pasture honey have protective effects on TJ following the challenge, and enhance post-challenge recovery of transepithelial resistance. Manuka honey had greater modulatory activity on TJ with increased concentration from 0.1–1%, and 1% concentrations of both honeys gave the greatest protective effects. Manuka honey appeared to have greater protective effects than pasture honey. Application of manuka honey (at 1% concentrations) to both the apical and basolateral sides of the MDCK cell monolayer significantly enhanced TJ tightness beyond the control. Dialysis of the honey confirmed that the high molecular weight fraction contained the active component. Diffusate fractions from either honey type had no effect on TJ. Artificial honey had no effect.

The effect of various honey concentrations on the proliferation of the 3T3-L1 fibroblast cell-line *in vitro* was investigated using MTT proliferation assays. Both manuka honey and pasture honey (0.25%) increased fibroblast proliferation. Artificial honey had no effect on fibroblast proliferation, indicating sugars have no role in mitogenic activity. This suggests that honey contains factors which directly promote cell division in fibroblasts.

An *in vitro* model was used to test whether honey-induced factors produced by peripheral blood mononuclear cells (PBMC) could activate fibroblast proliferation. These assays were performed to examine whether honey could have an indirect stimulatory effect on fibroblasts. Incubating fibroblasts with supernatants derived from honey-stimulated PBMCs (at 0.025% concentrations of honey) led to significant fibroblast proliferation. Low concentrations of honey (less than 0.1%) do not directly stimulate fibroblast proliferation, therefore factors produced by honey-stimulated PBMCs must promote fibroblast proliferation. A high molecular weight fraction of manuka honey obtained from dialysis contained the active component. The diffusate obtained by dialysis (containing sugars) had no activity.

To investigate whether honey could modulate the response of fibroblasts to an inflammatory agent, fibroblasts were exposed to honey for various times prior to and at the same time as IL-1 $\beta$ . Honey did not augment fibroblast proliferation when added at the same time as IL-1 $\beta$ . Prior incubation of fibroblasts with honey (0.25–1%) for 2 h before IL-1 $\beta$ -stimulation decreased the cell response to IL-1 $\beta$ , and this anti-inflammatory active component was of a high molecular weight.

It is proposed, on the basis of this *in vitro* study, that honey provides a neatly controlled therapy for optimising tissue repair, with potential for use in inflammatory disorders. It is the central thesis of this study that the stimulatory agent in honey induces cytokine production necessary for healing to occur, but that the oxidant species produced by these cells are effectively regulated by a second agent, thereby creating a feedback-regulated delivery system. The results presented in the current study suggest that honey can both stimulate and modulate cell activity, and show that honey interferes with a large number of regulatory steps in the inflammatory pathway, *e.g.* cell division, transcription, tight junction integrity, production of oxidant species. While the stimulatory activity was observed at lower concentrations, the modulatory effects required higher concentrations of honey. The dual ability for honey to stimulate moderate cellular activation in the absence of an immune stimulus, yet not augment mitogenic stimulation, and in some cases to modulate cell response to a mitogen, indicates that it will promote healing without setting up harmful inflammation.

If further experiments confirm this to be the case, honey will have potential for therapeutic application. The work also identifies some new areas of research, which if completed would further enhance the understanding of the role honey plays in tissue healing.

- **We have established through a survey of New Zealand honey varieties how they compare for antioxidant activity**

Molan, P. (2003) Report to the NBA on the TBG project developing an assay for the antioxidant activity of honey  
*Summary:* This project was undertaken to develop an assay for the antioxidant activity for honeys that would be suitable for routine use for the marketing of honeys with standardised levels of antioxidant activity. From the many different methods used in research work to measure antioxidant activity was chosen one that could be carried out easily and economically without the need for specialist instrumentation. This method involves measuring the loss of colour when the coloured ABTS stable free radical is scavenged by antioxidants. Laboratory investigation of this method with honey showed that there was interference from slow-reacting components of honey other than the useful fast-reacting antioxidants, so a modification of the method was developed which allowed measurement only of the useful fast-reacting antioxidants. The method was then adapted to being run on microtitre-plates in a microtitre-plate reader so that large numbers of samples could be run economically.

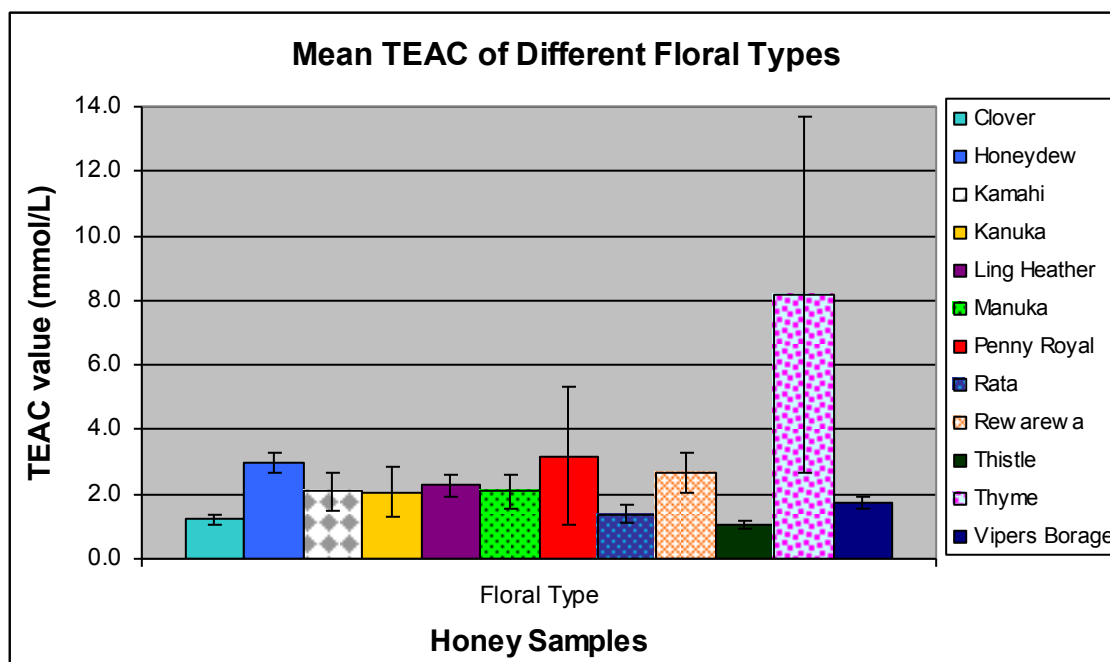
The optimum concentration range to work with was found for both the assay reagents and honey solutions. Checks were made of the stability of the assay reagents and the honey solutions. It was found that once honey has been diluted the antioxidant activity decreases on standing, presumably because hydrogen peroxide is produced, so assays need to be run soon after honey solutions are prepared. The reproducibility of the assay was determined by repeated measurements of samples many different types of honey, and coefficient of variance



ranged from <2% to 5%, the average being 2%. The recommended laboratory protocol for this assay is given in the Appendix to this report. A copy of the *Excel* spreadsheet containing all the formulae for calculating results from this assay accompanies this on disk.

A survey was run to find the relative antioxidant activity of the various types of honey that are produced commercially, testing at least ten samples of each type. The types with the highest activity were found to be thyme, honeydew and rewarewa. The variance within each type of honey was in some cases more than 100% from the mean value, showing the importance of assaying each batch rather than selling honey on the basis of being from a high-antioxidant source.

Preliminary work was also carried out investigating the stability of the antioxidant activity in undiluted honey of various types. Accelerated ageing of six different types of honey at 50°C for two weeks gave 0 – 15% loss of activity. Further work needs to be carried out on this.



- **We have discovered that some honeys have a super-protective antioxidant activity that binds iron to stop free radicals being formed**

Brangoulo, H. L.; Molan, P. C. (2011) "Assay of the antioxidant capacity of foods using an iron(II)-catalysed lipid peroxidation model for greater nutritional relevance." *Food Chemistry* **125**, 1126–1130.

**Summary:** The formation of free radicals by the iron-catalysed Fenton reaction is a major cause of oxidative damage in the body. Here a common assay of antioxidant capacity, inhibition of the  $\beta$ -carotene-linoleic acid model of lipid peroxidation, has been modified by the addition of ferrous iron (final concentration 36  $\mu\text{mol/l}$ ), which makes the rate of oxidation of the lipids occur twenty-five times faster. Such an assay can simulate the oxidative damage to membrane lipids and low density lipoproteins occurring in the body in the presence of free iron. It thus may be nutritionally more relevant than traditional chemical assays of antioxidant capacity, as it measures pre-emptive antioxidant activity, *i.e.* activity which prevents free radicals being formed in the first place. Pre-empting their formation is likely to be more protective than scavenging of free radicals. The relative antioxidant activity of some food products found using this new assay was very different from that found using a radical-scavenging assay. Vitamin C, at 280 mg/l, was found to be sixty times better than blackcurrant puree in

scavenging free radicals, but only one eighth as good as the blackcurrant puree in preventing iron-catalysed lipid peroxidation.

- **We have demonstrated that honey in the diet is healthier than sugar**

Chepulis, L.; Starkey (2008). "The long-term effects of feeding honey compared with sucrose and a sugar-free diet on weight gain, lipid profiles, and DEXA measurements in rats." *Journal of Food Science* 73(1): H1-H7.

**Summary:** To determine whether honey and sucrose would have differential effects on weight gain during long-term feeding, 45.2 mo old Sprague Dawley rats were fed a powdered diet that was either sugar-free or contained 7.9% sucrose or 10% honey ad libitum for 52 wk (honey is 21% water). Weight gain was assessed every 1 to 2 wk and food intake was measured every 2 mo. At the completion of the study blood samples were removed for measurement of blood sugar (HbA1c) and a fasting lipid profile. DEXA analyses were then performed to determine body composition and bone mineral densities. Overall weight gain and body fat levels were significantly higher in sucrose-fed rats and similar for those fed honey-fed compared with rats fed sucrose or a sugar free diet, but no other differences in lipid profiles were found. No differences in bone mineral density were observed between honey- and sucrose-fed rats, although it was significantly increased in honey fed rats compared with those fed the sugar-free diet.

Chepulis, L. M. (2007). "The effect of honey compared to sucrose, mixed sugars, and a sugar-free diet on weight gain in young rats." *Journal of Food Science* 72(3): S224-S229.

**Summary:** To determine whether honey, sucrose, and mixed sugars as in honey have different effects on weight gain, 40 6-wk-old Sprague-Dawley rats were fed a powdered diet that was either sugar free or contained 8% sucrose, 8% mixed sugars as in honey, or 10% honey freely for 6 wk. Weight gain and food intake were assessed weekly, and at completion of the study blood samples were removed for measurement of blood sugar (HbA1c) and a fasting lipid profile. The animals were then minced and total percentage body fat and protein measured. Overall percentage weight gain was significantly lower in honey-fed rats than those fed sucrose or mixed sugars, despite a similar food intake. Weight gains were comparable for rats fed honey and a sugar free diet although food intake was significantly higher in honey-fed rats. HbA1c and triglyceride levels were significantly higher in all sugar treatments compared with rats with a sugar free diet, but no other differences in lipid profiles were reported. No differences in percentage body fat or protein levels were reported.

Chepulis, L. M. (2007). "The effects of honey compared with sucrose and sugar-free diet on neutrophil phagocytosis and lymphocyte numbers after long-term feeding in rats." *Journal of Complementary and Integrative Medicine* 4(1): Article 8.

**Summary:** To determine whether honey and sucrose would have differential effects on levels of neutrophil phagocytosis after long-term feeding 36 2-month old Sprague Dawley rats were fed a powdered diet that was either sugar-free or contained 7.9% sucrose or 10% honey (honey is 21% water) ad libitum for 52 weeks. The percent of neutrophils exhibiting phagocytosis, and the percentage of leukocytes that were lymphocytes were then measured by flow cytometry after 52 weeks.

**Results:** Neutrophil phagocytosis was similar between sucrose- and honey-fed rats, and lower in rats fed the sugar-free diet (79.2%, 74.7% and 51.7 %, respectively). The percentage of leukocytes that were lymphocytes differed significantly between all three treatments, the levels being highest in honey-fed rats (53% vs 40.1% and 29.5% for sucrose- and sugar-free fed rats). In conclusion: Honey may have a beneficial effect on immune activity, possibly attenuating the decline seen in older age.

Chepulis, L. M.; Starkey, N. J.; Waas, J. R.; Molan, P. C. (2009). The effects of long-term honey, sucrose or sugar-free diets on memory and anxiety in rats." *Physiology & Behavior* 97(3-4): 359-368.

**Summary:** Sucrose is considered by many to be detrimental to health, giving rise to deterioration of the body associated with ageing. This study was undertaken to determine whether replacing sucrose in the diet long-term with honey that has a high antioxidant content could decrease deterioration in brain function during ageing. Forty-five 2-month old Sprague Dawley rats were fed ad libitum for 52 weeks on a powdered diet that was either sugar-free or contained 7.9% sucrose or 10% honey (which is the equivalent amount of sugar). Anxiety levels were assessed using an Elevated Plus Maze, whilst a Y maze and an Object Recognition task were used to assess memory. Locomotor activity was also measured using an Open Field task to ensure that differences in activity levels did not bias results in the other tasks. Anxiety generally decreased overall from 3 to 12 months, but the honey-fed rats showed significantly less anxiety at all stages of ageing compared with those fed sucrose. Honey-fed animals also displayed better spatial memory throughout the 12-month period: at 9 and 12 months a significantly greater proportion of honey-fed rats recognised the novel arm as the unvisited arm of the maze compared to rats on a sugar-free or sucrose-based diet. No significant differences among groups were observed in the Object Recognition task, and there appeared to be no differences in locomotor activity among groups at either 6 or 12 months. In conclusion, it appears that consumption of honey may reduce anxiety and improve spatial memory in middle age.

- **We have discovered the way in which the anti-inflammatory activity of honey works**

A paper is in the publication process: Bean, A., Cursons, R. T. and Molan, P. C. "Anti-inflammatory activity of honey found to work through inhibition of phagocytosis"

**Summary:** OBJECTIVES The mechanism of action of the widely reported anti-inflammatory properties of honey is unknown. It has been shown that honey decreases the release of reactive oxygen species from phagocytes, but the observed decrease may have been due to antioxidant components of the honey preventing oxidation of the probes used to detect them, rather than honey suppressing their production. We therefore looked for any direct effect of honey on phagocytosis

**METHODS** We investigated under the microscope phagocytosis by macrophages obtained by stimulation of immunocompetent THP-1 monocytes with lipopolysaccharide. Phagocytosis was assayed with various fluorescent particles: latex beads, zymosan, BCG mycobacteria and Escherichia coli.

**KEY FINDINGS** Honey inhibited the phagocytosis of all of the particles tested. Manuka honey at 0.5% gave more than 60% inhibition of phagocytosis of latex beads and 40-50% inhibition of phagocytosis of the microbial particles. It was not due to an osmotic effect of honey on the cells. Manuka honey at concentrations ranging from 0.125% to 2% gave a dose-dependent inhibition of phagocytosis of latex beads from 21% to 92% inhibition. Samples of sixteen other types of honey were tested (at a concentration of 0.25%) and some were found to also have an inhibitory effect but at a lower rate overall (mean inhibition 6.7%) than the forty-seven samples of manuka honey that were tested at the same concentration (mean inhibition 24.9%). There was a large variation in the degree of inhibition between the samples of manuka honey (inhibition ranged from 4% to 51%).

**CONCLUSIONS** As the inflammatory response can be initiated by phagocytosis, these findings provide an understanding of the anti-inflammatory action of honey.

- **We have discovered how honey works to rapidly remove attached pus and dead tissue from wounds**

A paper is in the publication process: Harcourt, N. R. and Molan, P. C. "Honey increases plasmin activity by suppressing production of PAI-1 by inflamed macrophages"

**Summary:** Honey, in the form of modern registered wound-care products, has become well established as a topical treatment to optimise wound healing, and is being noted to be especially effective in promoting wound

debridement. Plasmin activity is a key factor in the rate of debridement, and here we have shown in a cell culture model of inflammation that honey increases the quantity of plasmin activity, and that the mechanism by which this is achieved is through down-regulating transcription of the gene for plasmin activator inhibitor-1 (PAI-1). Using THP-1 monocytes that had been differentiated into macrophages by addition of phorbol myristyl acetate then inflamed by addition of lipopolysaccharide (LPS) we found that honey (at a concentration of 1%, the maximum osmolarity tolerated by the cells in culture) gave a 30% decrease ( $p < 0.01$ ) in expression of the gene for PAI-1. Measuring activity in culture medium from these cells confirmed that this translated into a 25% increase ( $p < 0.01$ ) in plasmin activator activity peaking 12 hours after addition of LPS, and that this was paralleled by a 21% increase ( $p < 0.01$ ) in plasmin activity. None of these effects were seen with a control of a mixture of sugars simulating the sugar content of 1% honey. Five different types of honey were tested and were all found to increase plasmin activity, but there were differences between the honey samples, even between multiple samples of the same floral type, in the degree of increase (21% – 103%) that they gave.