



The AF protein A clinical innovation of endogenous origin

Twenty years of Swedish research has led to the discovery of the Antisecretory Factor (AF), an endogenous protein with significance in secretory and inflammatory disease. The research has already resulted in medical foods with clinical use.



RESEARCH AND DEVELOPMENT

This brochure tells the story of 20 years of Swedish research leading up to the discovery of the endogenous AF protein, an antisecretory factor with the ability to regulate cellular fluid and ion transport over cell membranes in various organs of the body.

The effect of the AF protein on different types of secretion is very pronounced. Diseases where the transport of fluid is disturbed, for instance in gastrointestinal diseases, diarrhoea and Mb Ménière, have been studied to assess the clinical significance of AF protein. It has also shown marked anti-inflammatory properties that have been proven in studies of Irritable Bowel Diseases (IBD), rheumatic diseases and mastitis (inflammation of the breast). Recent American studies describe that the AF protein might even have an important role in regulating our immune system.

On the next few pages the story will be told of how a Swedish ban on antibiotics in animal feed led to the discovery and research of AF products with proven clinical effect in a varying number of diseases. Follow the journey from research to finished product and read more about the fascinating research and clinical trials that highlight the AF protein.

Pioneering research

Ban on antibiotics in animal feed and research in diarrhoeal diseases

In 1986 the use of antibiotics in animal feed was banned in Sweden, as one of the first countries in the world. The ban was due to an escalating antibiotic resistance developing in animals, similar to that seen in human antibiotic use. Antibiotics in animal feed are used to prevent for instance post-weaning diarrhoea in piglets. The ban resulted in an increased prevalence of diarrhoea. The suffering this caused and the increased number of deaths due to stress and infectious disease epidemics was disastrous and caused noticeable profit loss. To come to terms with the problem and possibly find an alternative feed, a joint scientific project was initiated, combining the expertise from veterinary medicine, microbiology, infectious disease and immunology.

Since the 1980's Stefan Lange and Ivar Lönnroth at the Department of Clinical Bacteriology at the Sahlgrenska University Hospital in Gothenburg, Sweden had been studying mice and cholera disease. They were searching for a substance that immunized mice and rats to diarrhoea caused by cholera toxin. It is well known that survivors of cholera, both rats and humans, develop resistance for a long time. Lange and Lönnroth discovered an endogenous system that reacted faster than the immune system, probably an endogenous substance prone to different types of stimulation. The resistance could be triggered by the addition of enterotoxins or by a combination of amino acids and sugars in defined proportions.

Through further research they discovered that a substance produced in the pituitary gland gave resistance to cholera toxin. The protein was named Antisecretory Factor, in short AF (Lange and Lönnroth 1984; Lange et al 1987). This regulatory protein affects the intestinal fluid and electrolyte balance in cell membranes in different organs. AF normalises intestinal fluid secretion, an important factor in the treatment of diarrhoeal disease.

Immediate results from novel AF-pig feed

The knowledge gained from the rat toxin studies and the discovery of AF protein was applied in



The ban on antibiotics in animal feed led to increased diarrhoea and mortality in piglets. Thanks to the introduction of a new kind of specially processed cereals with the ability to stimulate the production of an endogenous protein, the AF protein, these negative trends could be broken.

the search for a novel feed aimed at post-weaning diarrhoea in piglets. At first, feed with added amino acids and sugars was given to piglets to stimulate their own AF production. Urine samples showed that there was a correlation between the AF-level and diarrhoea. Samples from pigs without diarrhoea had a high level of AF whereas samples from pigs with diarrhoea had low levels of AF. The use of this specialised feed gave immediate effect, resulting in substantially lowered prevalence of diarrhoea and significantly fewer deaths in piglets.

From this point research began to develop a feed that in itself contained the proper amounts of amino acids and sugars, without the need for additives. A production process was created and in 1991 a feed intended for piglets and sows was introduced. The feed improved the piglets' natural resistance to gastrointestinal disease and has now become the dominating feed on the Swedish market.

Hydrothermal treatment yields specially processed cereals

The feed is produced through a patented process involving hydrothermal treatment. The cereals, oats and wheat, are first soaked in water, then heated. No additives are needed in the process. At the optimal temperature, the process is stopped and the cereals are dried and crushed. Amino acids and monosaccharides are produced when the starch is broken down, and enzymes are released. This enzymatically activated specially processed cereal (SPC) stimulates the endogenous production of AF protein.

The AF vision: Pharmaceuticals and Medical Foods

The encouraging results from the animal research led the researchers to look beyond and widen their scope to include use by humans. Three visions for the future were stipulated.

- 1) Develop an AF-pharmaceutical for humans by producing a synthetic peptide, analogous to AF protein.
- 2) Develop an analytical method for blood measurement of AF.
- 3) Produce medical foods using experience gained in animal feed. This project was given priority considering the time needed to develop human pharmaceuticals.

The initial tests with AF-products on humans

Since the mechanisms involved in AF are of general physiological nature and similar to all mammals, transferring experiences learned about AF from animals and applying it to humans was not as complicated as one could expect. The concept of AF-feed had also proved effective in calf, horse, dog and cat. The researchers began to test the piglet feed on themselves, preparing it as a porridge and baking bread. Also the gastroenterologists at Sahlgrenska University hospital became interested and began tests on patients with ulcerative colitis. All patients treated showed favourable results and responded with a significant increase of AF in the blood. This effect remained measurable for four weeks after the intake of piglet feed. These results showed that the same principle that worked in animals was also applicable to humans.

Europe's first medical food

Encouraged by the positive results seen on man the desire became to produce food based on AF protein. At this time EU was discussing the introduction of new dietary foods for special medical purposes (not to be confused with Functional Foods). The Commission Directive 1999/21/EC on dietary foods for special medical purposes was ratified in May 2000. The MagiForm® product range was the first to be approved according to the new legislation. The product range includes muesli, rusks and biscuits with AF stimulating effects. The range expanded to also include more potent stimulators in the form of pure SPC and Egg yolk powder B221®, a vehicle for exogenous administration of high dose AF protein.

Encouraging results with the AF-products in animals triggered the desire to develop pharmaceuticals and medical food for humans.

As some of the patients did not respond with increased levels of AF despite stimulation, the researchers started searching for a method of administering exogenous AF protein. This resulted in the Egg yolk powder B221®. It was discovered that hens fed with the AF-inducing feed laid eggs with AF-enriched yolks. For practical reasons an egg yolk powder was developed. Exogenous administration of AF protein, in the form of the egg yolk powder, is primarily used for acute treatment or for patients with severe disease or suspected low levels of AF. The SPC products on the other hand should be considered as maintenance treatment, stimulating the patients' own production of AF protein.

Chemistry and biology

Chemical and biological characterisation

In the early 1990's Ivar Lönnroth concentrated on chemical and biological characterisation of the AF protein. This work was completed in the mid 1990's. The protein is of medium size, 41 kDa, comprised of 380 amino acids with the active region located as a peptide of 8-38 amino acid length in the terminal N-region of the protein.

The purified protein was administered to rabbits and used to produce antibodies, in turn used to clone the protein coding gene. It is now possible to produce highly purified protein through microbiological methods as well as chemical synthesis.

The AF protein is fairly unique in that it is active both as a protein and as a peptide. It acts dually by stimulating the nerves in the intestine and by affecting the nerve signals regulating fluid transport. The protein has its own pharmacological effect and initiates production of endogenous defence mechanisms against secretion and inflammation. It is the most potent anti-diarrhoeal substance known and it has been proven to inhibit secretion caused not only by cholera, but also by a number of other known toxins, i.e. E.coli, Campylobacter, Clostridium difficile and okadaic acid, the blue mussel toxin.

Within a couple of hours the plasma level of AF rises quickly following exposure to diarrhoea stimulation. In healthy subjects AF is inactive and not of vital importance. It will however become activated upon stimulation from bacterial toxins. An elevated synthesis of AF can also be achieved by the ingestion of AF-inducing specially processed cereals (SPC).

Evidence suggests that the body has a biological memory of AF stimulation. This has been studied on healthy subjects. When given 1 g/kg bodyweight per day of AF inducing cereals administered 2-3 times daily one can expect to see significant elevation of AF levels. After 10-15 days of administration, levels will reach 1 AF-unit/ml plasma. Depending on the length of cereal intake the level will remain for a corresponding amount of time. A three-month break in the intake will cause a return to low AF levels. If the intake of AF-enhancing cereals is resumed, the level will reach significant values already after 2-3 days.

The peptide regulates the fluid and ion transport

over cell membranes in different organs of the body, probably by regulating the permeability in the channels responsible for water and ion transport. Thus, AF has a fundamental influence over diseases where secretion is of importance. It has effect in regulating fluid transport over the intestinal wall in e.g. Crohn's disease and fluid pressure in the inner ear in Ménière's disease, as well as other conditions caused by an imbalance in the fluid regulation in bodily tissues.

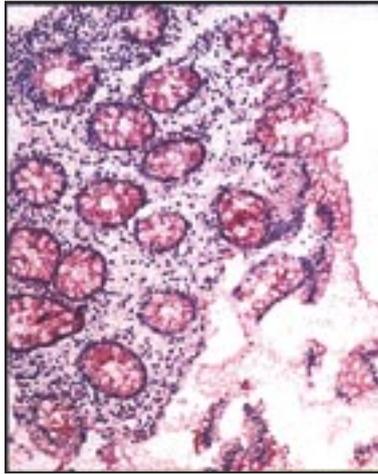
Aside from the highly potent antisecretory activity, AF protein also has important anti-inflammatory effects. The clinical significance of this activity has been shown in rheumatoid arthritis, inflammatory bowel diseases (IBD) such as ulcerative colitis and Mb Crohn, as well as other situations where inflammation plays an important role, e.g. in mastitis, common in breastfeeding women.

In 2001 the collected experiences from research and clinical trials was published in the renowned journal *International Review of Cytology* (Lange and Lönnroth, 2001).

A recent article by an American research group (Davidson and Hickley, 2004) suggests that AF protein may have a previously unsuspected role in regulating the immune system in the CNS. The authors believe that an increased knowledge about these and other mechanisms may lead to even further clinical applications of the AF protein.

AF is present in most tissues in the body

With immunohistochemistry techniques it has been shown that AF is present in most tissues in the body. Eva Jennische, at the Department of Anatomy and Cell Biology at Gothenburg University, has through studies shown that AF protein is located in three cell types; epithelial cells, lymphatic cells, especially leukocytes, and nervous cells. AF is expressed in the mucosal epithelium of the gastrointestinal tract, the respiratory organs, the urogenital organs and in pituitary cells, mainly endocrine cells in the pituitary gland. Under certain conditions also other white blood cells can express AF, i.e. during inflammatory response. AF protein has further been identified in central and peripheral nervous tissue, in neuronal cells and synaptic nerve terminals.



Immunoreactivity and histological changes after AF treatment. The photos above show tissues from a patient suffering from severe Crohn's disease before (left) and after (right) treatment with specially processed cereals (SPC).

As a result of the positive clinical effects of AF treatment of Ménière's disease, immunohistochemical analyses have been performed showing that AF can be localized to the Purkinje cells of the cerebellum, which probably are of importance for inhibitory vestibular functions. AF has also been found in the peripheral receptor organs, cochlea and the vestibular apparatus.

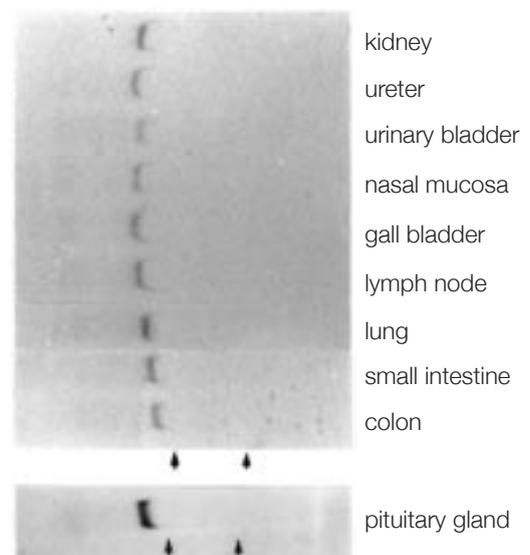
Research is ongoing to identify a true classical receptor for AF. So far, two binding proteins of interest have been identified, flotillin-1 and vestibule-1, who possibly can interact with AF. The researchers also believe that the GABA_A receptor can interact partially with AF.

Protein AF regulates water and ion transport over cell membranes in various organs. It is the most potent anti-diarrhoeal substance described. Alongside its antisecretory activity, it also shows pronounced anti-inflammatory properties. Further, new and potentially important immunological properties of protein AF have recently been described.

Stefan Lange developed a model (Lange, 1982) to measure the concentration of AF in different tissues achieved by measuring hypersecretion in the rat intestine by the sectioning of a 10-15 cm segment. The AF activity in man is measured by injecting purified AF from plasma intravenously in a rat stimulated with cholera toxin. An AF concentration

that inhibits 50% of the secretion in the loop is given an AF value of 1.0. The clinical studies have shown that an AF value exceeding 0.5 is correlated to an influence on diarrhoea. Northern blot has also been used in order to reveal mRNA for AF.

There is ongoing intensive research to find a direct method for determining the blood concentration of AF in humans, thereby avoiding indirect measurements in rat. Such a method would easily determine which AF level a certain person has. It is probable that a low AF level can be found in several diseases, whereas a high level is found in healthy subjects. A patient with a low concentration of protein AF could thereby start the treatment with a suitable AF product, and this without having to change his other medication.



AF protein in pig tissue. AF is present in almost all tissues in both animals and humans.

Antisecretory effects

Short intestines/Intestinal resections

After the encouraging results seen in animals and healthy subjects it was desirable to test the anti-diarrhoeal effects of the specially produced cereals (SPC) on patients with severe hypersecretion. Severe hypersecretion is experienced in patients with short intestines, often due to major intestinal resections. An open pilot study was designed under the guidance and expertise of Ingvar Bosaeus at the Department of Clinical Nutrition at the Sahlgrenska University Hospital (Lange, Bosaeus et al, 2003).

The study included eight patients with varying extents of intestinal resections due to Crohn's disease. The length of their small intestine varied between 80 and 400 cm. All patients suffered from chronic diarrhoea without concurrent bowel inflammation. Six healthy controls participated.

All subjects received 54 g SPC daily for 2 weeks. The daily dose was divided evenly over the day during at least three occasions in connection with mealtimes. Patient and control baseline diet and medications were kept unchanged.

Plasma AF activity was determined before, during and after the treatment period. The SPC diet increased AF activity in all controls. AF activity was increased from a mean of $0,28 \pm 0,37$ units/ml plasma prior to treatment to $1,28 \pm 0,23$ AF units/ml plasma after the two week treatment period ($p < 0,05$). Elevated levels of AF ($1,28 \pm 0,39$) were maintained during at least four weeks after intake of the SPC diet.

In the patients baseline values were low ($0,04 \pm 0,07$), rising during the treatment period to a mean of $0,66 \pm 0,45$ units. The two patients with the longest remaining small intestine, 300 and 400 cm, achieved elevated AF levels comparable to healthy subjects, i.e. over 1 unit/ml plasma. These patients also lowered the number of daily bowel movements. Analysis showed that in the patients there was a significant correlation between the length of the remaining small intestine and AF induction ($r=0.94$, $p < 0.01$) (Fig.1). It was concluded that a small intestine length of at least 1 metre is needed to induce AF and to maintain the elevated levels after the treatment with AF inducing diet.

Secretory diarrhoea due to carcinoid tumours

The pathology of diarrhoea in patients with short intestines is most likely due to multiple factors and for this reason it is possible that AF only influences the secretory component of the diarrhoea. To test this hypothesis a study was performed on patients with endocrine tumours (Laurenus et al, 2003). Patients with neuroendocrine tumours may suffer from severe diarrhoea, that may be both difficult to treat, treatment resistant and persistent despite an otherwise optimal treatment regime.

The aim of the study was to investigate if specially processed cereals (SPC) and Egg yolk powder B221® could induce AF-activity and if AF-therapy could

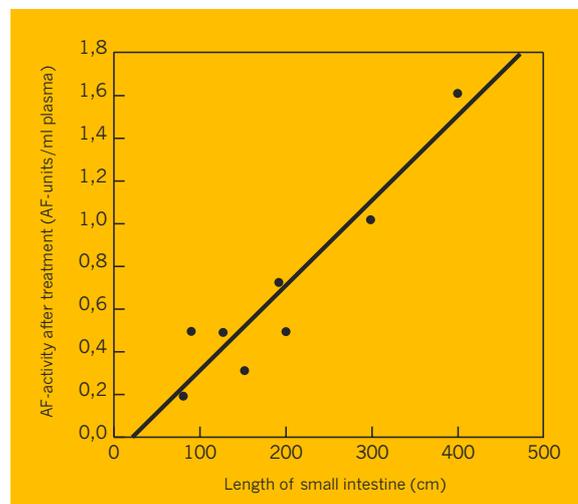


Figure 1. There is a significant correlation between the length of remaining small intestine and AF-level in patients with short intestines.

reduce the number of bowel movements. Egg yolk powder B221[®] was included in the study due to earlier positive results on diarrhoea seen in patients with intestinal resection. The treatment with AF products was administered as a complement to the patients' ongoing medical therapy.

Eight critically ill patients were included. Six patients with the midgut carcinoid syndrome and two with metastasizing medullary thyroid carcinoma (MTC) participated.

In an initial open part of the study all patients received Egg yolk powder B221[®] for 4 weeks. This was followed by randomisation to a double-blind crossover period with SPC and control cereals (CCs) without AF inducing ability for 6 weeks in a dose of 1 g/kg bodyweight divided into four doses daily.

Treatment with Egg yolk powder B221[®] resulted in a significant reduction of daily bowel movements ($p < 0,01$) (Table 1) and several patients reported firmer stools. The daily number of bowel movements was significantly lower during the SPC-period compared to the period with CC ($p < 0,05$) (Table 1).

All patients had low levels of AF-activity in serum at baseline. During treatment with Egg yolk powder B221[®], the mean level increased slightly. AF-activity was also significantly higher ($p < 0,05$) after SPC compared to the CC diet. In four of the five patients in which AF levels during treatment with SPC were measured, levels rose above 0,5 units/ml plasma. This level has in other studies correlated well with positive effects of diarrhoea treatment.

Although these patients had been given optimal medical therapy, an added positive effect could be seen with the addition of the AF treatment. Treatment with Egg yolk powder B221[®] was well tolerated by all patients. The two patients with MTC and fully intact intestines responded best to SPC-diet with the highest AF levels, reaching over 1 unit/ml plasma, after completed treatment.

The positive response to Egg yolk powder B221[®] indicates that AF protein has a local effect in the

intestine, directly interacting with the receptors and its binding proteins in mucosal cells. This could explain why systemic effects are seen after treatment with Egg yolk powder B221[®] despite that plasma levels do not exceed 0,5 units, the level at which SPC-induced endogenous AF shows significant activity. Therapy with Egg yolk powder B221[®] may hence be a suitable treatment alternative for patients with difficulties ingesting large amounts of specially produced cereals.

Ménière's disease

Since the AF products have such favourable results in secretory diseases of the gut, it was hypothesised if this could also be applied to other conditions where fluid imbalances are of importance or can be suspected.

It has been hypothesized that the symptoms of vertigo in patients with Ménière's disease somehow are related to impaired production and/or transport of endolymph resulting in an increased pressure on the inner ear. Patients suffering from Ménière's disease often experience vertigo, nausea and vomiting. The increased pressure in the balance nerve in the ear causes rotational vertigo. If the increased pressure is located to the auditory nerve the complications may be reduced hearing and tinnitus.

After several pilot studies a trial was performed at the department of ENT and Audiology at Sahlgrenska University Hospital under the guidance of Per Hanner (Hanner et al, 2004). The aim of the study was to examine if AF could be induced in cases of pathologically elevated pressure of the inner ear and to study if stimulated AF production can lessen the clinical symptoms of Mb Ménière patients.

24 patients with Mb Ménière, with a duration between ten months and thirty years, were included in the study. The patients received, in addition to their regular medication, a SPC-diet at a dosage of 1 g/kg bodyweight and day, for 2-4 weeks. The patients kept a diary over their subjective and auditive

Table 1. Number of bowel movements during treatment compared by paired t-test

Test period	Mean±SD	n	p
Baseline	5,6±2,6	7	<0,01
Egg yolk powder B221 [®]	4,2±2,4	7	
Control cereals	4,0±1,9	5	<0,05
SPC	2,6±1,3	5	

symptoms, and frequency, duration and characteristics of vertigo. Baseline tone and speech audiometry and neurological status was determined as well as AF plasma level before and after the treatment period.

The concentration of AF in plasma varied between 0-0,6 units before treatment and 0-1,7 units after treatment (Table 2). 83% of the patients showed an increase of AF above 0,5 units after treatment, the level at which clinical improvement of secretory

Symptoms were either reduced or in complete remission in more than half of the patients with severe Ménière's disease who were treated with a simple, inexpensive and well tolerated treatment.

diarrhoea has been shown in previous studies. In 17% of the patients, little or no increase of AF was seen and they did not experience any improvement of the clinical symptoms either. In 29% of the patients a significant increase of AF was registered but without clinical response.

In 12,5 % of the patients hearing ability returned to normal levels and vertigo was completely eradicated. In 54% vertigo was improved. The improvements noted ranged from complete remission to remaining light and diffuse feelings of dizziness without previous attacks of rotatory vertigo.

Despite the positive results seen on half the treated Mb Ménière patients the researchers were interested in continuing studies to deduce why some of the patients had no clinical response to AF-inducing cereals.

Studies using the immunohistochemistry technique have shown that AF can be found both in the central nervous system and peripheral receptor organs, which could open for the possibility of influencing both central and peripheral regulatory functions.

Treating Mb Ménière with Egg yolk powder B221®

As the study above showed, there are some patients that are non-responders when treated with AF inducing specially processed cereals (SPC). Due to the positive results of Egg yolk powder B221® in the treatment of secretory diarrhoea, it was tested on Ménière's disease as well (Hanner et al, 2003).

AF-inducing SPC was tested during a two month period on a 67 year old woman with a thirty year history of Mb Ménière. She had frequent attacks lasting for 8 hours up to three days with nausea, vertigo, diarrhoea and vomiting. When the SPC diet did not improve neither the duration of the attacks nor the severity, she was given Egg yolk powder B221® at a dose of 2 g five times daily to see if this exogenous administration of AF in high concentrations could offer positive clinical results.

On day 18 of the treatment the patient was completely relieved of her symptoms and her Ménière status was improved from 6 to 1 (American Academy of Otolaryngology). After six months of symptomatic relief the patient lowered the dosage of Egg yolk powder B221® with the result that her attacks with rotatory vertigo returned. Returning to the original dosage level led to immediate improvement without the need for further medication. No improvement however could be seen on her right-sided hearing impairment.

The results show that patients who do not respond to treatment with AF-inducing cereals can be favourably treated with Egg yolk powder B221®. To stimulate the body's own production of AF it is likely that an introduction of AF-inducing cereals in connection with or as a follow-up to Egg yolk powder B221® treatment will be found valuable.

Table 2. Mean plasma AF-concentrations in patients with negative and positive effect of treatment, compared by paired t-test.

Test period	Mean±SD	n	p
Before treatment, neg eff	0,19±0,17	10	ns
Before treatment, pos eff	0,25±0,25	13	
After treatment, neg eff	0,67±0,53	11	<0,01
After treatment, pos eff	1,28±0,30	13	

Anti-inflammatory effects

Inflammatory bowel diseases (IBD); Mb Crohn and ulcerative colitis

A study was performed to see if AF inducing cereals could promote endogenous AF production in patients with ulcerative colitis and Crohn's disease, and to determine if an elevated AF level was correlated to clinical improvement of intestinal function (Björck et al, 2000).

The experimental group consisted of 26 patients given AF inducing cereals as muesli, bread and/or pasta in addition to their regular diet during 30 days. A control group of 24 received a similar diet without the AF inducing properties. Patients were asked to keep a diary over bowel symptoms, and plasma levels of AF and rectal biopsies were evaluated before and after treatment.

Results showed that the patients receiving active treatment significantly improved intestinal function

correlating to increased plasma AF levels ($p < 0,001$). Elevated AF levels were sustained one month after treatment. No effect was seen in the placebo group (Fig. 2). A significant accumulation of AF in intestinal epithelial cells of the active treatment group was seen with colonic biopsies.

Subjective ratings of clinical symptoms according to VAS were significantly improved ($p < 0,05$) in the group receiving active treatment (mean +34,6%) compared with placebo (mean 15,4%) (Fig. 3).

Due to the findings of this study the surgeons have begun AF-treatment of more patients undergoing GI-surgery in which through experience the outcome is known to lead to reduced intestinal function.

Treatment is started very early, in some cases the first day, by giving Egg yolk powder B221[®] dissolved in ordinary juice. This is a way of administering high amounts of AF directly to the intestines without hav-

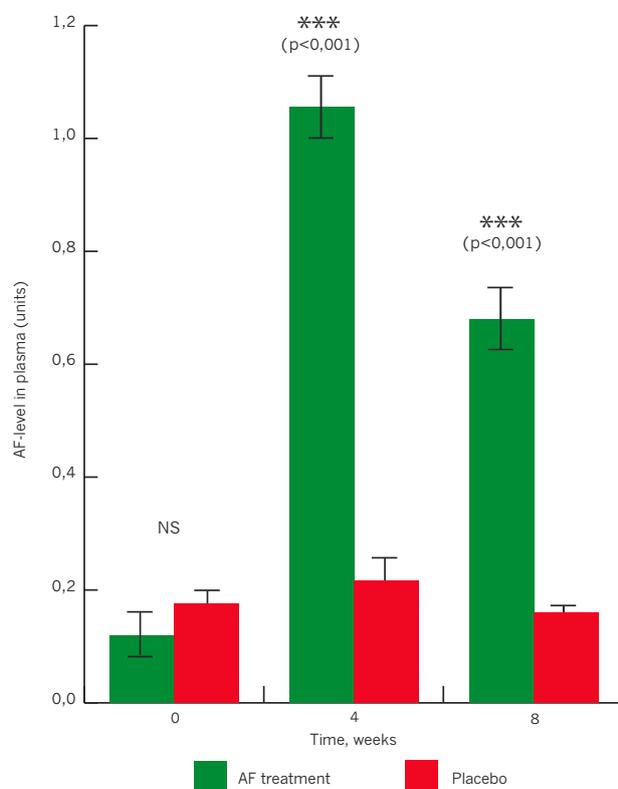


Figure 2. Patients treated with specially processed cereals (SPC) had significantly higher AF-levels in plasma compared to placebo. SPC-treated patients also got a significantly better intestinal function.

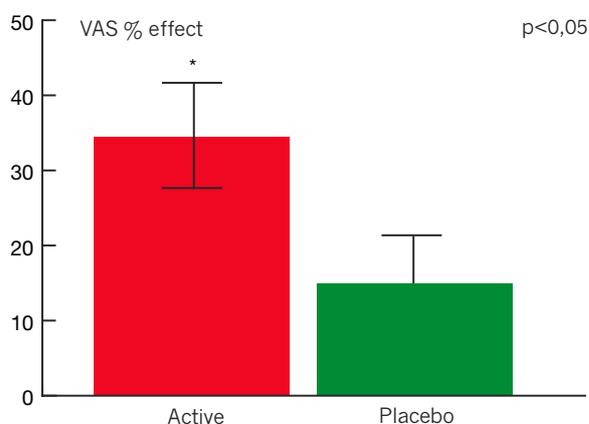


Figure 3. AF-treated patients showed a significant improvement in subjective symptoms as compared to the placebo group.

ing the body producing it first. Most of the time the administration of Egg yolk powder B221® can be discontinued after 5-10 days and changed to a maintenance dose of AF inducing cereals, since the desire is to stimulate the patients endogenous production of AF.

IBD treatment in children

Recently the first pilot study on children suffering from Crohn's disease and ulcerative colitis was reported (Finkel, Bjarnason et al, 2004). The results show improved clinical symptoms and that treatment with specially processed cereals (SPC) is safe and well tolerated. These positive results will be followed up with a randomised double-blind study.

Treatment of severe Mb Crohn

Prior positive results from AF-treatment on Crohn's patients encouraged an open study on a group of six patients with severe, prolonged treatment resistant Crohn's disease (Shafazand et al, 2003). Patients were treated with AF inducing cereals during three months. Clinical symptoms, blood samples, endoscopic and histological examination, CDAI (Crohn's Disease Activity Index) and quality of life (SF-36) were evaluated.

A continuous and significant improvement of clinical symptoms and quality of life measurements was seen (Fig 4 and 5) as well as histological improvement. Findings from this and earlier studies show the anti-inflammatory activity of the specially processed AF inducing cereals. It is most likely that it is the anti-inflammatory activity that is of greatest importance in the treatment of Crohn's patients.

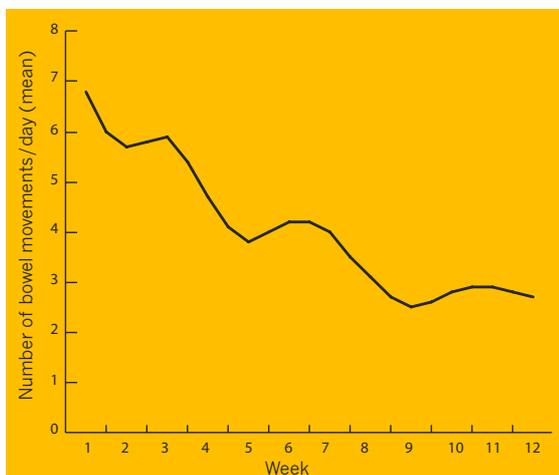


Figure 4. A continuous and significant improvement was seen in the reduction of daily bowel movements.

Treatment of acute onset of ulcerative colitis with Egg yolk powder B221®

The influence of an additive treatment of orally administered AF, through Egg yolk powder B221®, was studied in patients suffering from acute onset of ulcerative colitis. Patients were given 2 g Egg yolk powder B221® four times daily for 14 days. A control group was given egg yolk powder without AF inducing ability (Eriksson et al, 2003).

A total of 20 patients, 10 in each group, fulfilled this prospective, randomised, double-blind study. The intake of Egg yolk powder B221® was used as

It is most likely that the antisecretory effect of AF in ulcerative colitis is of importance only after the reduction of inflammation.

an additive treatment to conventional medication, which also included parenteral or enteral nutrition.

Earlier trials performed on patients with chronic IBD, endocrine diarrhoea and diarrhoea due to intestinal resection showed varying, but significant effects on the number of bowel movements following induction of AF. The aim of this study was primarily to measure the histological effects on the intestinal epithelium. In addition other laboratory parameters of inflammation and clinical effect were studied.

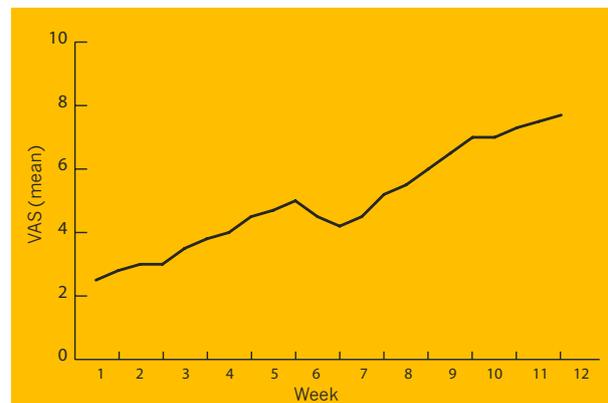


Figure 5. The patients also experienced a continuous and significant improvement in quality of life measurements.

During AF treatment, a reduction of inflammation in mucosal biopsies received from the mid-rectum was found. The secretory effect was not significantly different between groups. It was therefore concluded that the antisecretory effect of AF in ulcerative colitis is of importance only after the reduction of inflammation. The observation that AF treatment partially restored the intestine supports this hypothesis. It is likely that the antisecretory effect can be achieved by administering AF treatment in higher doses or through AF inducing cereals (SPC), triggering the endogenous production of AF protein.

Prevention of mastitis with AF

Inflammation of the mammary gland is a painful and common problem for breastfeeding women. Problems can be of a passing inflammatory nature or it may involve long term problems with severe symptoms. In cases where infection cannot be

AF-inducing cereals (SPC) can probably increase AF-levels in breast milk, thereby preventing sub-clinical mastitis. This condition increases the risk of transferring virus from an HIV-infected mother to her child.

identified, inflammation may be the cause. A new theory has presented the possibility that sub-clinical mastitis may also increase the risk of transmitting HIV-1 from mother to child.

Earlier studies had shown that women in developing countries had measurable amounts of AF in breast milk. The amounts exceeded those measured in women in the industrial world. It was therefore decided to perform a randomised double-blind study on breastfeeding mothers in Sweden at the Karolinska Hospital (Svensson et al, 2004).

12 women were given AF inducing cereals (SPC) 3-7 days post partum. 16 women acted as controls, given cereals without AF-inducing ability. In the treated group only one woman developed mastitis com-

pared to six women in the control group. Three women in the control group were diagnosed with mastitis twice and one woman three times ($p=0,0086$). The only woman who developed mastitis in the treatment group had misunderstood the dosage and only ate SPC on weekdays, not weekends as well.

AF levels in breast milk were measured before and after treatment. The median AF level was significantly higher in the AF treated group, 1,1 AF unit/ml breast milk (0,7-1,25), compared to the control group, 0,1 AF unit/ml breast milk (0,0-0,25) ($p<0,0001$). The median AF level in women with mastitis was significantly lower, 0,0 AF units/ml breast milk (0,0-0,1), than the level in women who did not have mastitis, 0,5 units/ml breast milk (0,2-1,1) ($p=0,017$). The one woman in the active treatment group who did develop mastitis had the lowest AF level in the group.

The study showed that an AF level in breast milk above 0,5 units/ml is protective against mastitis. This result is concurrent with the results seen in the animal studies, in which the same level is enough to protect the offspring from developing diarrhoea (Lange and Lönnroth, 2001). These results can be of great importance to developing countries since diarrhoeal diseases are still a great problem. It is of course also of great importance if AF-inducing cereals (SPC) have the ability to reduce sub-clinical mastitis and thereby may reduce the risk of HIV-1 being transferred from an HIV-positive mother to her child.

AF treatment of rheumatoid arthritis

As earlier studies had shown that AF-inducing cereals had a positive effect on symptoms and disease processes in diseases of inflammatory nature, it became of interest to study what effect AF-inducing cereals (SPC) may have on the activity of rheumatoid arthritis (Mörck et al, 2003).

In a randomised, double-blind, placebo controlled study patients with active rheumatoid arthritis were allocated to either SPC or control cereals (CC). The cereals were given at a daily dose of 0,5 g/ kg body-weight, divided into three doses daily, for 12 weeks. This dosage is lower compared to normal dosage (1g/kg bw). Prior medication remained unchanged (methotrexate and salazopyrin) and no patients were currently on cortisones. CRP, DAS 28 (Daily Activity Score measured in 28 joints) and HAQ (Health Assessment Questionnaire) was recorded at baseline, and after 4 and 12 weeks of treatment. 16 patients in the treatment group and 18 in the control group followed through.

The results show a significant increase of AF-levels in the SPC treated group (Table 3). Baseline comparisons and after 12 weeks treatment showed a significant difference in CRP between groups. CRP was in-

creased by 66±29% in the control group, whereas it was reduced by 4±12% in the active treatment group (p<0,05). No difference between groups could, however, be seen in status and symptoms of the joints.

Table 3. Mean plasma AF-concentrations compared by paired t-test.

Test period	Mean±SD	n	p
Before treatment, SPC	0,2±0,1	5	ns
Before treatment, control	0,2±0,1	4	
After treatment, SPC	1,1±0,1	5	<0,001
After treatment, control	0,1±0,1	4	

New areas of development

Travel prophylaxis

The AF protein can be induced in healthy individuals and the elevated levels are sustained up to four weeks after treatment with AF-inducing cereals (SPC). It is most likely that the body has a biological memory of AF production.

People living in “dirty” environments have a naturally higher level of AF protein, probably due to an over exposure to bacterial toxins and other factors that have the ability to stimulate the AF system. It has therefore been considered if it could be possible to trigger endogenous defence mechanisms and immunise travellers against diarrhoeal diseases when visiting problem areas. No clinical trials have been performed yet, but the theory is that a high AF level could prevent diarrhoeas caused by enterotoxins.

Future clinical studies and areas of development

Which diseases are caused by inflammatory reactions and which are predominantly of secretory nature? The answer to these questions will lead the way to new areas of research and applications.

In all studies so far patients have continued with their current medication and diet. As treatment with AF-products is gentle and well tolerated there are several areas in which clinical research are of interest. Some examples of diseases in which fluid imbalances and/or inflammation plays a part and may become of interest for AF-treatment are listed below.

Secretory diseases/processes

Diabetes (AF in pancreatic cells)
 diarrhoea in HIV patients
 pre-op/acute op. (improve nutritional status)
 Cystic fibrosis
 Mb Ménière
 oedemas (cerebral oedema)

Inflammatory diseases/processes

Mucositis (following cytotoxic treatment)
 intestinal fistulae
 proctitis
 microscopic colitis
 Mb Bechterew
 rheumatoid arthritis
 Mb Crohn in children
 Irritable bowel syndrome

Treatment

Dosage

SPC: Normal dosage of AF-inducing cereals (SPC) is 1 g/kg bodyweight daily, divided into 2-3 occasions. When treating children the dose may need to be increased and when treating elderly it may need to be reduced. The dosage should be introduced step by step and reach full dosage after 4-6 days. The cereals can be eaten with yoghurt or boiled to a porridge.

Egg yolk powder B221®: Normal dosage is 2g 5 times daily. The powder is blended with a cold liquid, e.g. water or juice, preferably using a whisk or a blender.

Egg yolk powder B221® is the recommended AF treatment for patients who have difficulties eating large amounts of specially processed cereals. Studies also indicate that patients not responding to treatment with AF inducing cereals may respond to Egg yolk powder B221®.

To stimulate the endogenous production of AF is has been favourable to introduce AF inducing cereals (SPC) during treatment with, or as a follow-up treatment towards the end to, Egg yolk powder B221®.

For seriously ill patients or in patients with suspected low AF-levels, treatment with Egg yolk powder B221® quickly raises AF-levels directly in the intestine. Most often the patients initially treated with Egg yolk powder B221® for 5-10 days can thereafter be transferred to the AF-inducing SPC, in order to stimulate the body's own production of AF.

20 g of SPC is equally potent to 2 g Egg yolk powder B221®.

Expected treatment outcome

A positive therapeutic response can normally be seen after 10-15 days of SPC treatment and within hours after ingestion of Egg yolk powder B221®. After termination of the treatment levels will decline again after a few weeks. If treatment is resumed, AF levels will rise within a matter of days, indicating a biological memory for AF production.

Side effects, interactions and precautions

AF products have been eaten by tens of thousands of individuals and no side effects of medical nature have been reported. A few patients have experienced occasional constipation, but this discomfort can be reduced by a step-by-step introduction of the treatment.

There are no reported interactions with concomitant medications. This is of importance as the patients should continue with their regular medical treatment. Neither SPC nor Egg yolk powder B221® should replace or change the patients' current medication. Persons with celiac disease or gluten intolerance should not be treated with AF inducing cereals and persons with allergy to eggs should not be treated with Egg yolk powder B221®.

References

- Björck, Bosaeus, Ek, Jennische, Lönnroth, Johansson and Lange, *Food induced stimulation of the antisecretory factor can improve symptoms in human inflammatory bowel disease: A study of a concept*, Gut, 2000; 46:824-829
- Davidson and Hickey, *Distribution and immunoregulatory properties of antisecretory factor*, Laboratory Investigation, 2004 Mar; 84(3):307-319
- Eriksson, Shafazand, Jennische and Lange, *Effect of Antisecretory Factor in Ulcerative Colitis on Histological and Laborative Outcome: A short Period Clinical Trial*, Scand J Gastroenterol, 2003; 38: 1045-1049
- Finkel, Bjarnason, Lindblad and Lange, *Specially Processed Cereals: A clinical innovation for children suffering from inflammatory bowel disease?*, Scand J Gastroenterol, 2004; 39:87-88
- Hanner, Jennische and Lange, *Antisecretory Factor: A clinical innovation in Ménière's disease?*, Acta Otolaryngol, 2003; 123:779-780
- Hanner, Jennische, Lange, Lönnroth and Wahlström, *Increased antisecretory factor reduces vertigo in patients with Ménière's disease: a pilot study*, Hearing Research, 2004; 4803:1-6
- Lange, *A rat model for an in vivo assay of enterotoxic diarrhoea*, FEMS Microbiol Lett, 1982; 15:239-242
- Lange and Lönnroth, *Passive transfer of protection against cholera toxin in the rat intestine*, FEMS Microbiol Lett, 1984; 24:165-168
- Lange, Lönnroth and Skadhauge, *Effects of the antisecretory factor in pigs*, Pflügers Arch, 1987; 409:328-332
- Lange and Lönnroth, *The Antisecretory Factor: Synthesis, anatomical and cellular distribution and biological action in experimental and clinical studies*, International Review of Cytology, 2001; vol 210:39-75
- Lange, Bosaeus, Jennische, Johansson, Lundgren and Lönnroth, *Food-induced antisecretory factor activity is correlated with small bowel length in patients with intestinal resection*, APMIS, 2003; 111:985-988
- Laurenius, Wängberg, Lange, Jennische, Lundgren and Bosaeus, *Antisecretory factor counteracts secretory diarrhoea of endocrine origin*, Clinical Nutrition, 2003; 22(6):549-552
- Mörck, Ek, Jennische, Laurenius, Tarkowski och Lange, *Anti-inflammatorisk effekt av kosttillskott med specialprocessade cerealier vid reumatoid artrit – en dubbel-blind pilotstudie*, Posterpresentation vid Riksstämman, 2003
- Shafazand, Eriksson, Jennische och Lange, *Födoinducerad ökning av antisekretorisk faktor förbättrade det kliniska tillståndet hos sex patienter med svår Crohns sjukdom*, Posterpresentation vid Riksstämman, 2003
- Svensson, Lange, Lönnroth, Widström and Hanson, *Induction of antisecretory factor in human milk may prevent mastitis*, Acta Paediatrica, 2004, in press

AS-FAKTOR AB

Box 30192, SE-104 25 Stockholm, Sweden. Phone 0046-8-657 42 70. info@as-faktor.se www.as-faktor.se