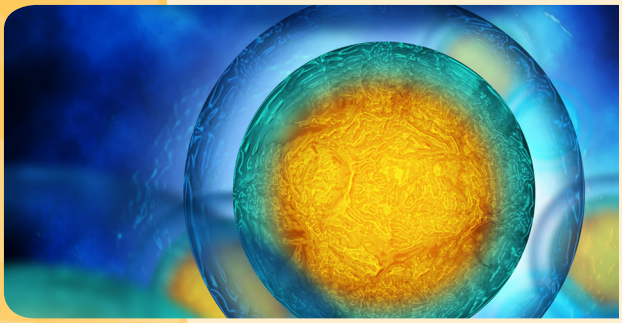
MODES OF ACTION

EPICOR®

Biochemical Markers Observed in EpiCor® Studies



Much of the research on EpiCor fermentate has used the "Gold Standard" of science – randomized, double-blind, placebo-controlled human trials with proven clinical endpoints published in peer-reviewed, Medline-indexed journals. A summary of these human clinical trials can be found in Embria's "*Summary of Human Clinical Trials on EpiCor.*"

These clinical trials demonstrate EpiCor provides statistically significant improvements in the health and wellness of healthy adult subjects by showing reduction of cold/flu, allergy and mild constipation symptoms. This paper reviews documented biomarkers which may be involved in these clinical effects.

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Scientific summary written for international product developers, industry experts, academia, and health care professionals. Not for distribution to consumers.

EPICOR RESEARCH:
REFERENCES FOR BIOMARKERS & CLINICAL ENDPOINTS LISTED IN THIS SUMMARY

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SUMMARY OF BIOMARKERS & CLINICAL ENDPOINTS

Categories		Statistically Significant Biomarkers & Clinical Endpoints *(Unless noted in EpiCor Research)*	EpiCor Research *(Numbers Refer to References)*		
			In vitro	*In vivo*	Human
Immune Health	Lymphocytes	Proliferation of B lymphocytes	10		
		Proliferation of T lymphocytes	10		
		Decreased nasal smear lymphocytes during allergy season			3
	Leukocytes	Decreased eosinophils during allergy season			3(T)
	Cytotoxicity	Activity of NK Cell	10		5
	Antibody Modulation	Increased production of secretory IgA			2,3
		Increased production of IgG			2(T)
		Decrease production if IgE			2(T),3(T)
	Production of Immuno-active compounds (cytokines)	Decreased TNF-a	12		
		Decreased IFN-ɣ	10	8	
		Decreased IL-8	10,12,13		
		Increased IL-10	10		2(T)
	Induction of activation markers	Increased CD80 on B lymphocytes	10		
		Increased CD86 on B lymphocytes	10		
		Increased CD25 (IL-2 receptor) on CD3+CD56+NK Cells			5
		Increased CD69 on CD3+CD56+NK Cells			5
		Decrease serum T Cell indicating homing			5(IE)
		Decrease serum NK Cells indicating homing			5(IE)
	Cold/Flu	Reduced incidence of cold/flu symptoms			1,4
		Reduced duration of cold/flu symptoms			1,4(T)
		Reduced hoarseness, stuffiness and feelings of weakness			1
	Allergy	Reduced nasal congestion and runny nose			3
		Reduced incidence of allergy symptoms			3
		Decreased need of rescue medication			3
	Inflammation	Decreased carrageenan-induced localized inflammation		8	
		Reduced collagen-induced inflammation (arthritis)		8	
		Reduced histamine-induced inflammation			6
		Reduced neutrophil production of ROS	10		
	Antioxidant Effects	Relatively high ORAC (614 umol TE/g)	11		
		Increased antioxidant power in serum (Cap-e)	11		
		Antioxidant protection of red blood cells	11		5
Gut Health	Mild Constipation	Reduced digestive discomfort including bloating and feelings of fullness			7(N)
		Improved stool consistency			7(N)
		Improved stool frequency			7(T)
	Prebiotic properties	Increased *Anaerostipes, Akkermansia muciniphila & Bacteroidetes*			7(W)
		May help balance Bacteroides/Firmicutes ratio			7(W)
		Increased SCFA (butyrate)	12,13		
		Increased lactobacilli	12,13		
		Qualitative modulation of bifidobacteria	12,13		
	Gut Morphology/ inflammation	Decreased coliforms, clostridia, staphylococci and facultative anaerobes	10		
		Prevented heat-induced reduction of villi height		8	
		Prevented heat-induced reduction in total mucosal thickness		8	
		Prevented heat-induced increase in vesicle concentration & in vesicle diameter		8	
		Prevented heat-induced increase in white blood cell counts (WBC)		8	
		Decreased LPS in blood of normal non-stressed animals		8	
		Prevented heat-induced increase in LPS in blood		8	
Eythrocyte Health		Increased hematocrit			2

T = Trend | IE = Indirect Evidence | N: Not statistically significant at all time points | W: Statistically significant within group

Biomarkers mentioned in this summary are from published human research conducted on healthy adult subjects unless labeled as *in vivo* or *in vitro*.

1. INNATE AND ADAPTIVE IMMUNE SYSTEM

- **Significantly increases NK Cell activation in less than two hours post-consumption versus placebo[5]**

PHYSIOLOGY: NK cells are a type of white blood cell that provides a rapid immune response within the innate immune system. In many cases the NK cells activity means an adaptive response isn't even needed. However, if an adaptive response is needed, NK cells are also known to play a role in the slower acting but more specific adaptive immune response.

- **Significantly increases secretory salivary immunoglobulin A (sIgA) versus placebo[2,3]**

PHYSIOLOGY: sIgA is the antibody that serves as the first line of defense in the mucosal immune barrier. sIgA is found in mucous secretions, including tears, saliva and colostrum as well as other secretions from the genitourinary tract, gastrointestinal tract, prostate and respiratory epithelium. It is a major component of the body's adaptive immune system in defense against foreign organisms and is an important link between the adaptive and innate immune systems. Recent science shows that sIgA may also play an important role in gastrointestinal health and overall immunity through reduced inflammation in the digestive tract.

- **Increases B cell activation[10] (*In vitro*)**

PHYSIOLOGY: B cells are an essential component of the human adaptive immune system. Their principal function is to make antibodies against antigens.

2. ENVIRONMENTAL ALLERGIES

- **Significantly decreases lymphocytes in nasal smears in EpiCor group versus placebo[3]**

PHYSIOLOGY: Increased lymphocytes would be expected in the nasal smears of healthy people exposed to environmental allergies.

- **Strong trend toward reduction of eosinophils in EpiCor group versus placebo[3]**

PHYSIOLOGY: Nasal eosinophils are commonly elevated in healthy people exposed to environmental allergies7

- **Total white blood cell count remained constant in the EpiCor group, whereas there was a mild trend towards an increase in white blood cells in the placebo group[2]**

PHYSIOLOGY: White blood cells tend to proliferate in people due to the onset of seasonal allergies.

- **Significantly reduces microvascular inflammatory responses to histamine-induced skin inflammation, and significantly reduced subjective scores of irritation at the inflamed sites[6]**

PHYSIOLOGY: White blood cells tend to proliferate in people due to the onset of seasonal allergies.

- **Trend toward relative decrease in serum IgE versus placebo[2,3]**

PHYSIOLOGY: Pollen binds to IgE antibodies present on the mast cells of allergy sufferers. The mast cells, and similar cells like basophils activate to release chemicals, including histamine, into the blood vessels and tissues. The binding of histamine to histamine receptors produces inflammation in surrounding tissues and causes nerve stimulation, leading to symptoms of itchy, watery eyes, sneezing, runny nose, and itching of the nose and throat.

- **Significantly decreases PGE2 levels[8] *(In vivo)***

PHYSIOLOGY: Prostaglandins, including PGE2, are also produced in large amounts during allergen exposure. PGE2 promotes immune inflammation.

- **Significantly decreases Nerve Growth Factor (NGF)[8] *(In vivo)***

PHYSIOLOGY: Studies have shown increased production of NGF in people with sensitivity to environmental allergens.

3. ANTIOXIDANT

- **Significantly increases serum antioxidant protection seen two hours post-consumption[5]**

PHYSIOLOGY: Antioxidants are known to protect cells against damage by free radicals.

- **Significantly reduced reactive oxygen species (ROS) produced by neutrophils[10] (*In vitro*)**
- **Increases protection from oxidative challenges in polymorphonuclear (PMN) cells when they are prevented from making inflammatory molecules[11] (*In vitro*)**

PHYSIOLOGY: Reactive oxygen species formation in PMN cells is frequently used to evaluate antioxidant capabilities and is a useful model for assessment of overall anti-inflammatory versus immune supportive properties of an ingredient. ROS formation can cause damage to healthy cells.

- **Increases antioxidant power in a dose-dependent manner with increased dosage in the Cap-e assay using red blood cells and also with polymorphonuclear cells(PMN)[11] (*In vitro*)**

PHYSIOLOGY: Based on the in vitro CAP-e assay (which measured the ability of red blood cells to resist oxidative damage when challenged with a pro-oxidant like hydrogen peroxide), and assays using PMN cells, research EpiCor shows a clear and linear dose-response.By showing increased effect with increasing doses, this proves that it is EpiCor that is providing antioxidant protection.

4. GUT HEALTH/RELATIONSHIP BETWEEN GUT HEALTH AND IMMUNITY

- **Significantly increases secretory salivary IgA (sIgA) versus placebo[2,3]**

PHYSIOLOGY: sIgA is one of the most important antibodies in the gastrointestinal immune system. It is a first line of defense in the immunological barrier against pathogens by modulating immune exclusion, regulating the intestinal microecology, inducing immune tolerance and inhibiting inflammation and allergic reactions, as well as performing other functions.

- **May increase the level of *Anaerostipes*, a genus containing acetate- and lactate-consuming and butyrate-producing bacteria[7]**

PHYSIOLOGY: Butyrate is well known for it's intestinal health benefits.

- **May increase the level of *Akkermansia muciniphila*[7]**

PHYSIOLOGY: *Akkermansia muciniphia* has been found to help with proper gut functioning, and reduced levels of this bacterium have been correlated with metabolic disorders as well as with inflammatory conditions like IBS.

- **May decrease *Blautia* and *Roseburia*[7]**

PHYSIOLOGY: A higher relative adundance of *Blautia* and *Roseburia* are seen in constipation-associated IBS.

- **May decrease the Firmicutes/Bacteroidetes ratio[7]**

PHYSIOLOGY: A two-fold increase in the ratio of Firmicutes to Bacteroidetes has been found in C-IBS patients when compared to healthy individuals.

- **May increase *Prevotella*[7]**

PHYSIOLOGY: A lower incidence of the *Prevotella* species has been hypothesized to be associated with a low-fiber diet and insufficient plant-based food consumption, and so to be a major cause of dysbiosis in the gut of constipated patients. The significant increase in Prevotella may partly explain EpiCor's positive effects on stool frequency and consistency.

- **May increase *Bacteroides*[7]**

PHYSIOLOGY: *Bacteroides* are significantly less abundent in constipated subjects.

- **Clinical results demonstrated significantly reduces microvascular inflammatory responses to histamine-induced skin inflammation, and significantly reduced subjective scores of irritation at the inflamed sites[6]**

PHYSIOLOGY: Histamine induces inflammation, and is associated with allergic conditions. The skin model may reflect what happens when histamine is present in the mucosa

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- **May prevent heat-induced increase of LPS endotoxin in the blood[9] *(In vivo)***

PHYSIOLOGY: LPS endotoxin in blood is known to be deleterious to good health.

- **May prevent heat-induced increase in white blood cell counts[9] *(In vivo)***

PHYSIOLOGY: High white blood count is associated with inflammation.

- **May prevent heat-induced increase in vesicle concentration and in vesicle diameter[9] *(In vivo)***

PHYSIOLOGY: Increased vesicle concentration and diameter are indicative of decreased erythrocyte health.

- **May prevent heat-induced reduction in total mucosal thickness[9] *(In vivo)***

PHYSIOLOGY: Reduced mucosal thickness indicates a reduction in gut wall integrity.

- **Prevented heat-induced reduction of villi height[9] *(In vivo)***

PHYSIOLOGY: Reduced villi height indicates a reduction in gut wall integrity.

- **May modulate the gut microflora, increasing the proportion *bifidobacteria* and *lactobacilli* (B&L)[12,13] *(In vitro)***

PHYSIOLOGY: B&L are lactic acid-producing bacteria constituting a major part of the intestinal microflora in humans and other mammals. An important role of the microflora, from the point of view of the host, is to colonize and thus help prevent colonization by harmful microganisms.

- **May increase production of butyrate[12] *(In vitro)***

PHYSIOLOGY: Butyrate is the major energy source for the intestinal epithelial cells, is considered to have anti-inflammatory effects, and has been studied for its role in nourishing the colonic mucosa.

- **May decrease production of proinflammatory cytokines[12] *(In vitro)***

PHYSIOLOGY: The change in composition of the microbial community of the gut caused a reduction in the production of inflammatory cytokines IL-8 and IL-1b in a model of the gut lining.

5. INFLAMMATORY RESPONSE

- **IL-10 trended up after EpiCor consumption, while it remained unchanged in the placebo group[2]**

PHYSIOLOGY: IL-10, also known as human cytokine synthesis inhibitory factor (CSIF), is an anti-inflammatory cytokine.

- **Clinical results demonstrated significantly reduced microvascular inflammatory responses to histamine-induced skin inflammation, and significantly reduced subjective scores of irritation at the inflamed sites[6]**

PHYSIOLOGY: Histamine induces inflammation, and is associated with allergic conditions. The skin model may reflect what happens when EpiCor is in contact with the intestinal epithelial cells.

- **Significantly decreases Nerve Growth Factor (NGF)[8] *(In vivo)***

PHYSIOLOGY: Increases in NGF are associated with discomfort and pain related to inflammatory responses because it impacts mast cells and afferent neurons.

- **EpiCor fed to mice had significantly lower arthritis scores versus placebo[8] *(In vivo)***
- **Arthritic mice showed significant increase in Interferon Gamma (IFN-γ) levels in control mice versus mice fed EpiCor[8] *(In vivo)***

PHYSIOLOGY: IFN-γ is one of the primary endogenous mediators of inflammation and immunity. It is known to be a pro-inflammatory cytokine and it was increased in this mouse model.

- **PGE2 levels were significantly reduced after consumption of EpiCor[8] *(In vivo)***

PHYSIOLOGY: A major mediator of the localized inflammatory response in this model is the proinflammatory prostaglandin PGE2, which has a role in other medical conditions as well. For example, in autoimmune diseases such as rheumatoid arthritis, PGE2 has a pro-inflammatory function.

- **Rat paw edema severity was significantly reduced after consumption of EpiCor[8] *(In vivo)***

PHYSIOLOGY: Edema, or swelling, is a major clinical endpoint associated with this inflammatory model.

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