

HOFSETH
BioCare®

DEVELOPMENT PROGRAM OF A SALMON PROTEIN HYDROLYSATE - PROGO®



MINIMAL PROCESSING - MAXIMUM OUTCOME

OUR FACILITIES

All located on the Norwegian west-coast



Hofseth
Raw material supplier



Hofseth Aalesund
Raw material supplier



Seafood Farmers
Raw material supplier



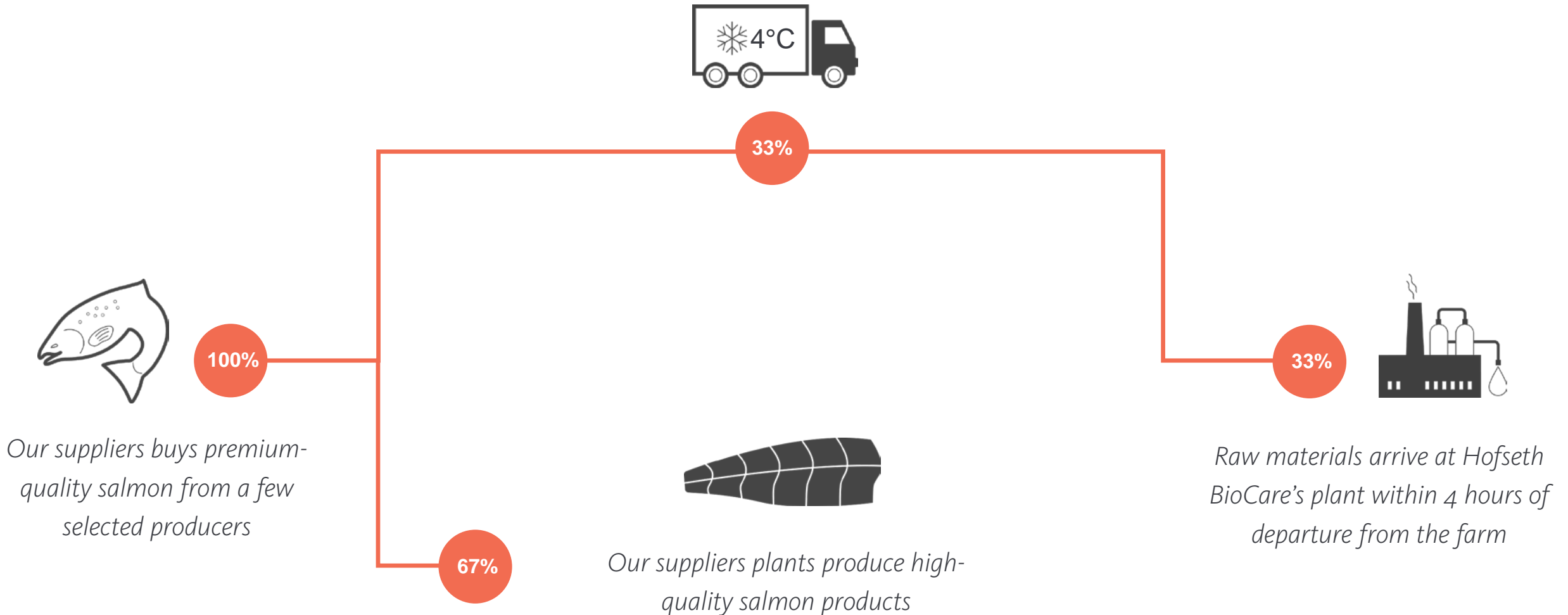
Hofseth BioCare
Hydrolysis plant



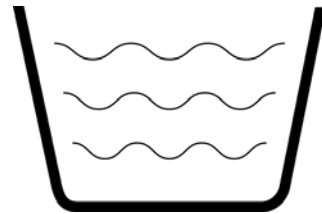
Hofseth BioCare
Spray-drying plant

SUSTAINABLE PROCESS

> ENHANCED VALUE



Raw material + Enzymes + Water



**SALMON OIL
OMEGO™**



**SALMON PROTEIN
PROGO™**

*(Spray dried at our dedicated
Berkåk Facility)*



**SALMON CALCIUM
CALGO™**



**PHP
PETGO™**

33%

*Our patented enzymatic hydrolysis
process gently extracts the product
from raw material at Midsund*

Agenda

- > **Specifying a new ingredient**
- > Animal Based Lead Generation
- > Human Efficacy Clinical Trials
- > Genetic Assay Lead Refinement
- > Developing New Markets

ProGo 

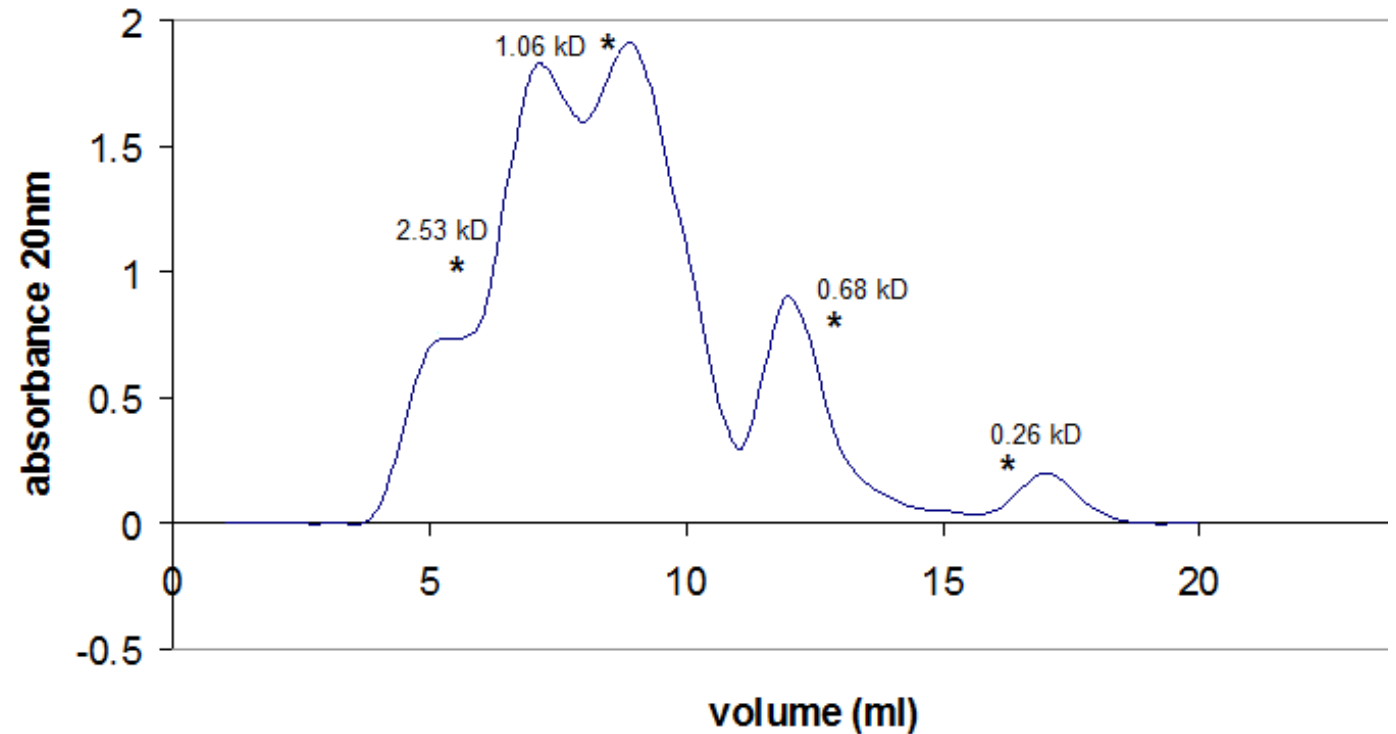


Physical Properties

- > Low fish taste and no bitterness
- > Makes a clear solution in water at 15% w/w (high solubility expands market scope)
- > GPC analysis shows a consistent narrow small peptide band – 50% within 1500-2000 D
- > Hundreds of individual peptides present
- > Hypoallergenic by ELISA analysis
- > Good blend of essential amino acids

Molecular Weight distribution by GPC analysis

HP GPC Superdex 75HR10/30 - Sample - SP Dry



SPECIFICATION

MICROBIOLOGY

Total aerobic microbial count	< 10000 CFU/g
<i>Escherichia Coli</i>	Absent/g
<i>Enterobacteriaceae</i>	< 10 CFU/g
<i>Listeria Monocytogenes</i>	Absent/25 g
<i>Salmonella</i>	Absent/25 g
<i>Staphylococcus Aureus</i>	Absent/g
Yeasts and moulds	< 100 CFU/g

CHEMICAL CHARACTERISTICS

Color	Light yellow
pH (2 % solution)	6.0 – 7.0
Moisture	< 5 %
Total Nitrogen (TN)	> 15 %
Alpha Amino Nitrogen (AN)	3.0 – 3.8 %
Protein (N x 6.25)	> 95 %
Fat	< 1 %
Ash	< 4.0 %

AMINO ACIDS ACTUAL QUANTITY (G/100 G)

Alanine	8.3
Arginine	7.2
Aspartic Acid	9.1
Cysteine	0.4
Glutamic Acid	13.7
Glycine	15.6
Histidine + Glutamine	2.1
Isoleucine	2.9
Leucine	5.6
Lysine	6.8
Methionine	2.5
Phenylalanine	3.4
Proline	7.3
Serine + Asparagine	4.9
Threonine + Citrulline	3.0
Tryptophan	0.5
Tyrosine	2.0
Valine	3.8

TYPICAL MOLECULAR WEIGHT DISTRIBUTION (G/100G +/-0.5G)

> 10000 dalton	0
1000 - 3000 dalton	44.1
< 1000 dalton	47.5

MINERALS AND ELEMENTS

Calcium	340 mg/kg
Copper	1.2 mg/kg
Iron	3.5 mg/kg
Magnesium	490 mg/kg
Potassium	19 g/kg
Sodium	11 g/kg
Zinc	8.1 mg/kg
Phosphorous	7.9 g/kg

Arsenic (inorganic)	< 0.1 mg/kg
Cadmium	< 0.05 mg/kg
Mercury	< 0.05 mg/kg
Lead	< 0.05 mg/kg

WHO-PCDD/F-TEQ	< 2 ng WHO-PCDD/F-TEQ/kg
WHO-PCB-TEQ	< 3 ng WHO-PCB-TEQ/kg
WHO-PCDD/F-PCB-TEQ	< 5 ng WHO-PCDD/F-PCB-TEQ/kg

SHELF LIFE

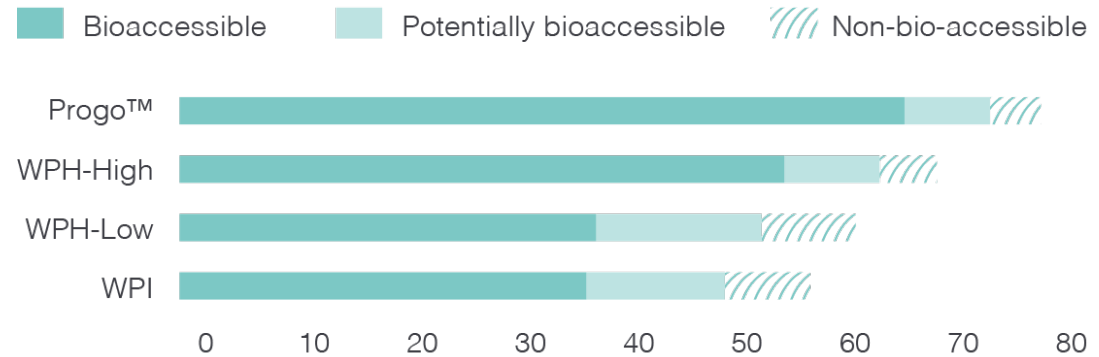
4 years from manufacturing date in unopened package.

ProGo[®] TIM-1 study

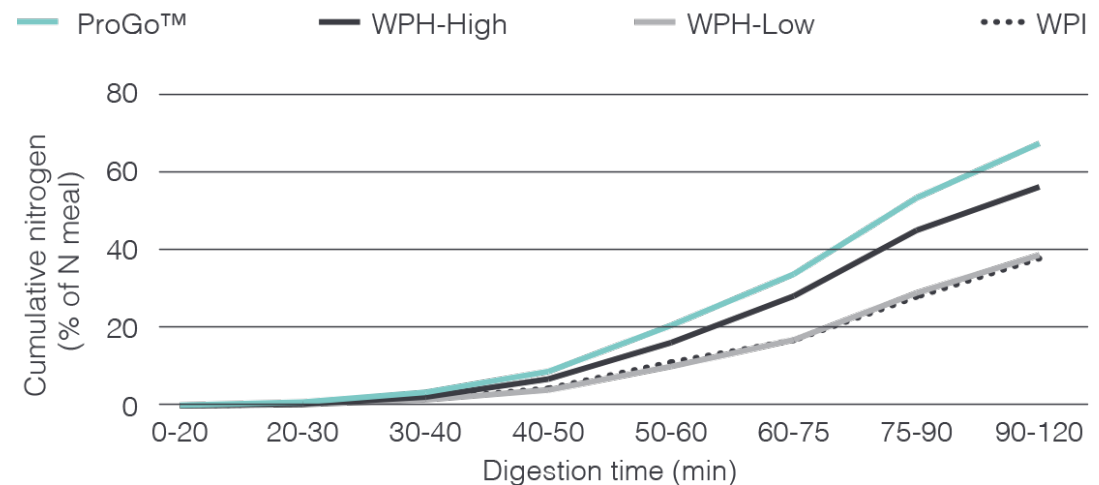
- > The TIM-1 Model was used to initially study uptake in human GI tract
- > Maximum uptake (> 80%) was in the upper GI tract
- > More than 98% digestible

Functional Foods in Health and Disease 2014; 4(5):222-231 Prof. Sylvie F. Gauthier, Laval University, Québec

Bioaccessibility



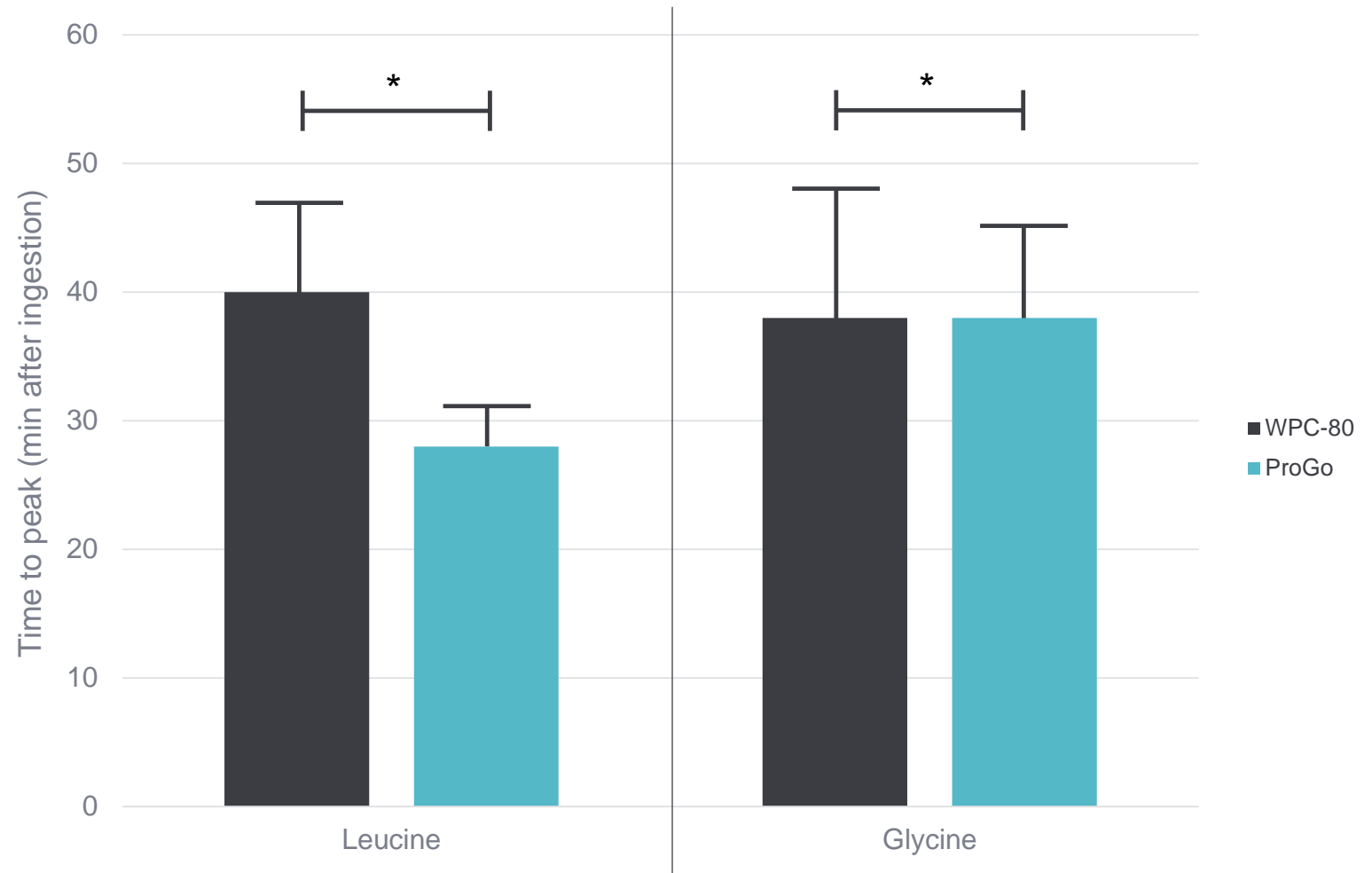
Digestion times



ProGo® - Follow up human uptake study

- > A randomized, double-blinded cross-over study comparing increase in blood leucine and glycine levels
- > A 15 minute faster time to peak blood concentration of leucine was observed for ProGo® versus WPC-80 yet glycine had a similar time to peak.
- > Leucine-trigger concept was reason for study as ProGo® as lower leucine content than in Whey

Journal of Nutrition and Food Technology v3.2 April 2017 – Prof Truls Raastad, Norwegian Sports Institute



* significantly different between protein drinks (p<0.05)

Agenda

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- > **Animal Based Lead Generation**
- > Human Efficacy Clinical Trials
- > Genetic Assay Lead Refinement
- > Developing New Markets

ProGo 



ProGo[®] - Animal Based Lead Generation

- > Animals Typically Used by us:
- > Sprague Dawley Rats
- > General multipurpose model used in safety and efficacy testing, aging, nutrition, diet-induced obesity, oncology
- > Senior Pointer Dogs
- > General model for studying reduced metabolism in geriatrics and health debilitated individuals
- > Occasionally we have used guinea pigs as well

Lead Generation Plan

Phase 1

- Treat animal with daily dosing of Y mg/g body weight (maximum tolerable dose based on comparative studies)
- Collect blood/serum at Day 0, Day X, Day 10X
- Assay for various biomarkers in serum that are up-regulated / down-regulated

Phase 2

- Repeat as dosing down study (0.1Y – 0.0001Y)
- Identify the selected biomarkers for further studies

Phase 3

- Repeat with second species
- Identify best biomarkers/disease of interest from above for further trials

ProGo[®] - Animal Based Leads

- > Sprague Dawley Rats
- > Fed 20% equivalent protein as ProGo
- > 4 weeks
- > Serum collected at start and end
- > Senior Pointer Dogs
- > Fed 20% equivalent protein as ProGo
- > 6 weeks
- > Serum collected at start and end

Leads Found

Phase 1

- Various biomarkers in serum showed changes in both animal models
- In Senior Pointer Dogs also noted a sharp increase in energy and movement

Phase 2

- Repeated as a dosing down study
- Identified several other biomarkers for followup studies

Phase 3

- Repeated with Guinea pigs
- Three biomarkers for two potential chronic conditions showed cross-species and dose-dependent effect
- Increased concentration of ferritin and decrease in transferrin coupled with an increase in serum hemoglobin indicated potential iron uptake effect
- Increased serum levels of adiponectin indicated a potential “fat burn” weight control effect.

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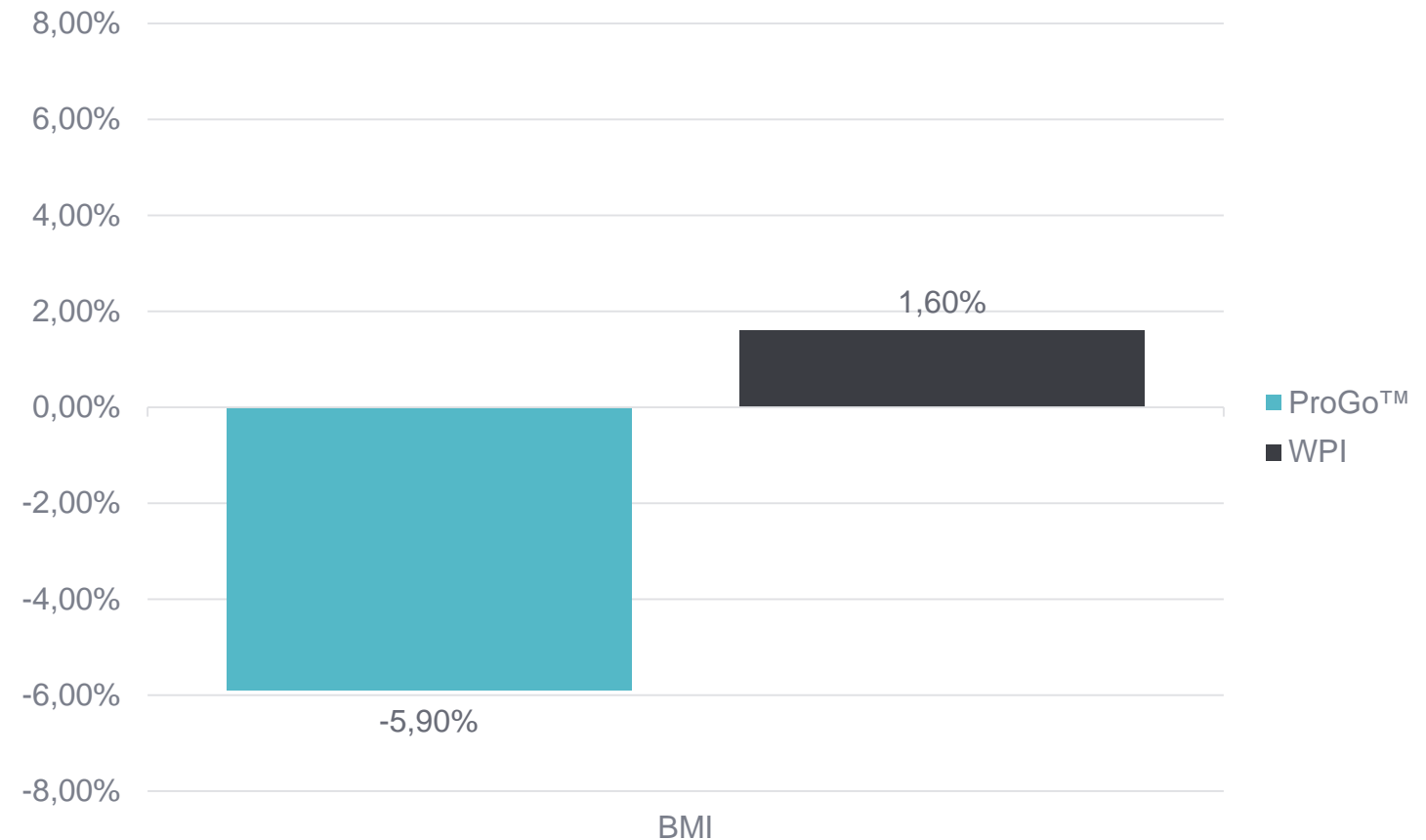


ProGo[®] - Follow up of Adiponectin result shows BMI Decrease in Humans

- > Obesity is a major global health problem with over 3 million adults dying each year from obesity related complications
- > 16 g / day ProGo[™] treatment for 6 weeks showed a 5.9% decrease in Body Mass Index
- > Normal diet and exercise was maintained throughout the trial
- > Five biomarkers showed a positive response
- > Salmon protein hydrolysate powder in supplemental doses may be a useful tool in the long-term management of obesity.

Journal of Obesity & Weight Loss (2016) **6(1)** 296

Change in BMI and metabolism serum biomarkers after 6 weeks of treatment



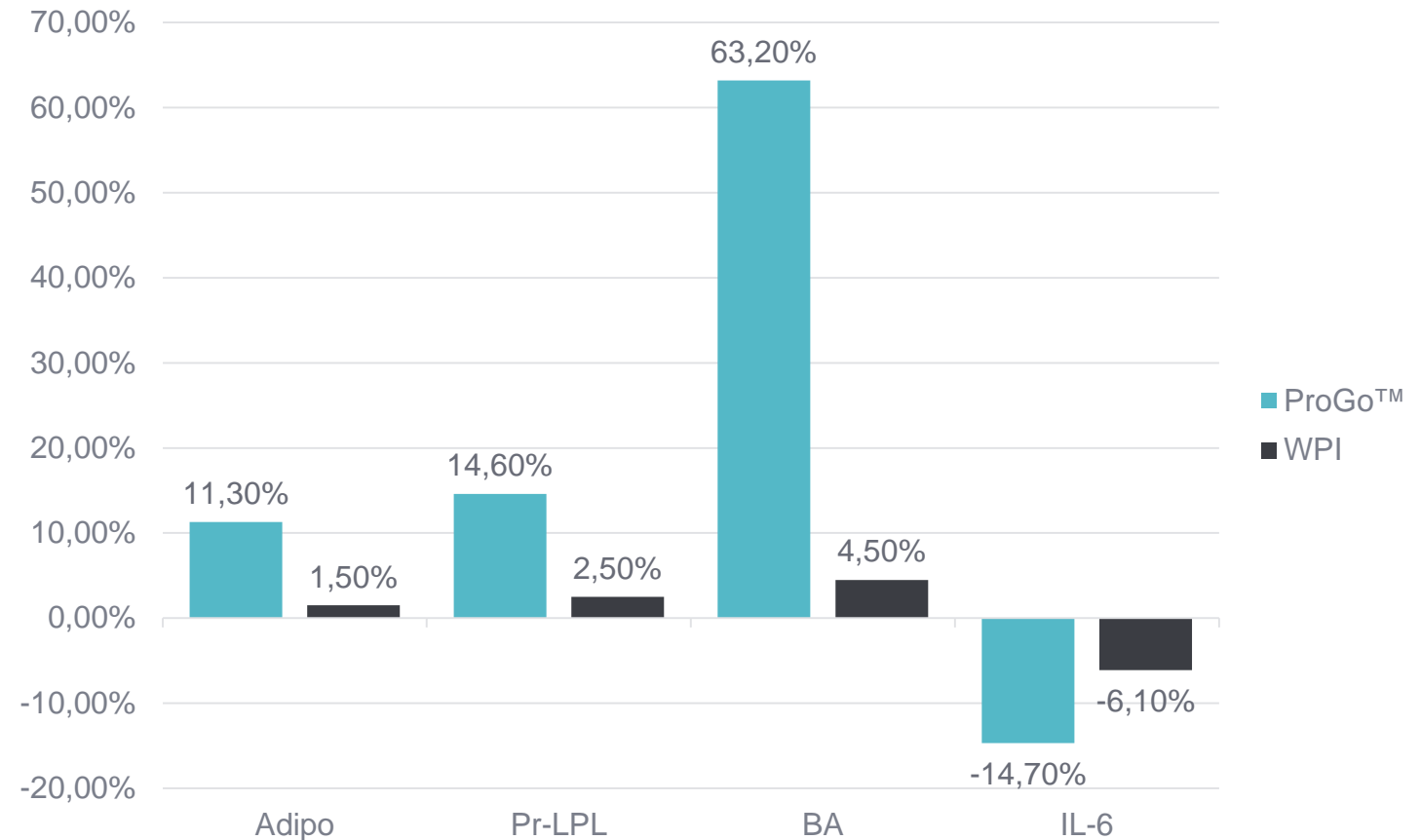
* All results above were significantly different between protein drinks ($p < 0.05$)

ProGo[®] - Follow up of Adiponectin result shows BMI Decrease in Humans

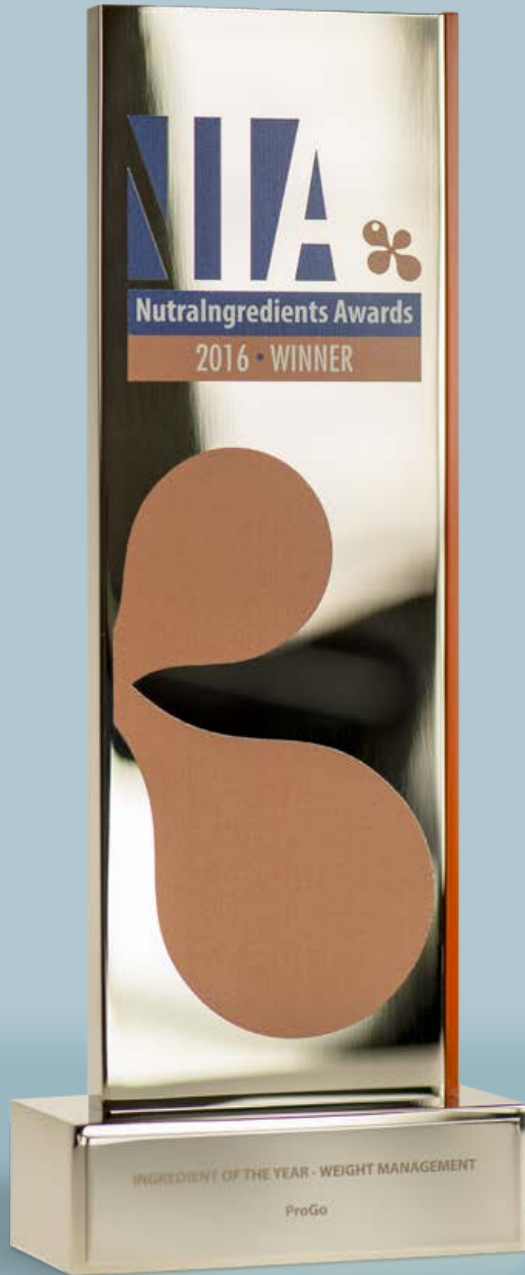
- > Adiponectin is an adipose-derived plasma protein with anti-atherogenic and insulin-sensitizing activities associated with metabolic syndrome. Elevated serum levels indicative of effectiveness of therapy
- > Preheparin Lipoprotein lipase (Pr-LPL Mass) is a lipolytic enzyme. Increased levels reflect improved insulin sensitivity
- > Bile acids are essential for solubilization of dietary fats and increased presence reduces plasma and liver TG levels
- > The reduced Interleukin 6 (IL-6) level can be indicative of an improvement in underlying inflammatory metabolic disorder.

Journal of Obesity & Weight Loss (2016) 6(1) 296

Change in BMI relevant serum biomarkers after 6 weeks of treatment



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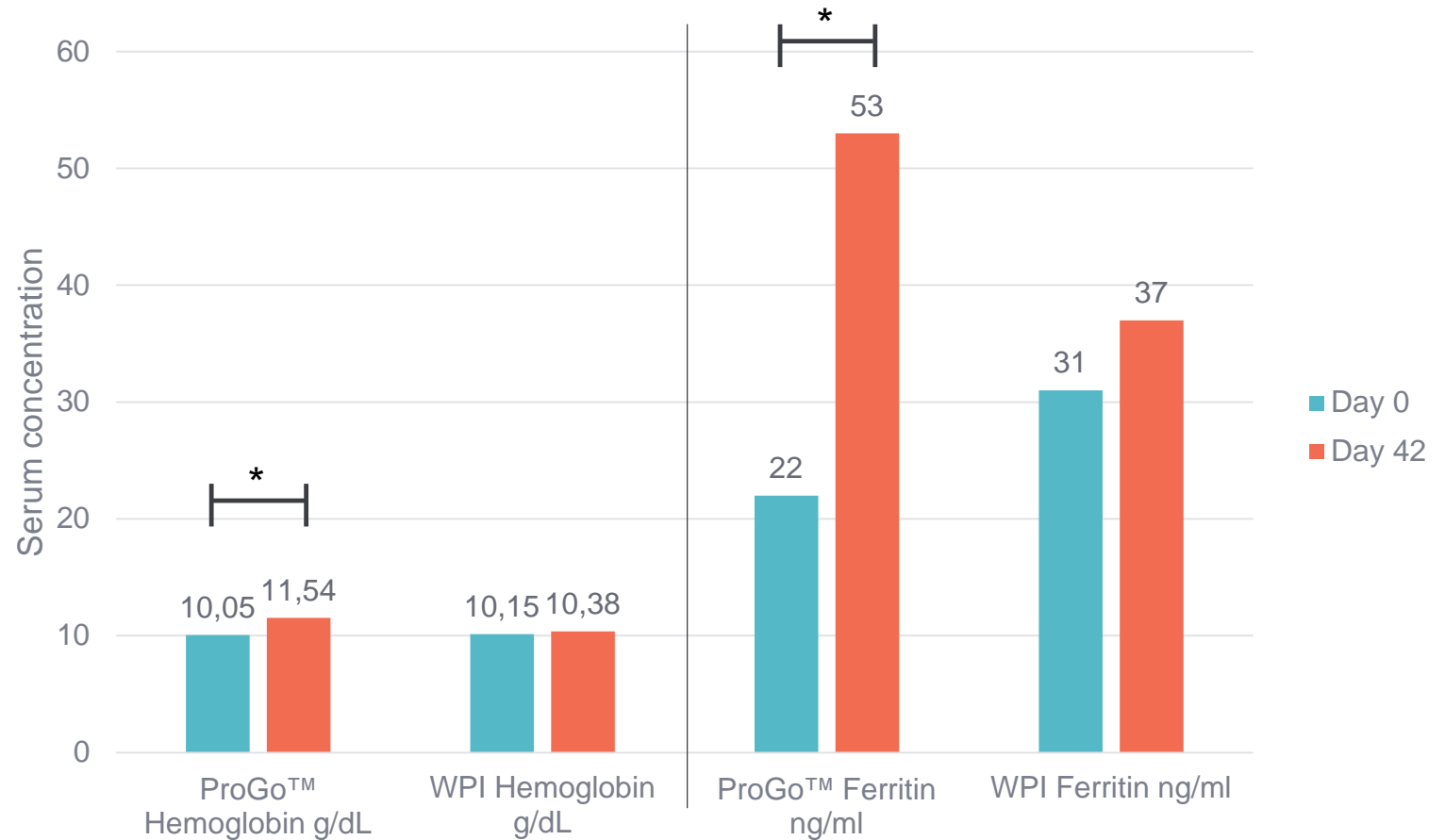
NUTRAINGREDIENTS AWARDS 2016

Ingredient of the Year - Weight Management

ProGo® Follow up of Ferritin result shows Hemoglobin Increase in Humans

- > Iron deficiency anemia is the most common micronutrient deficiency globally
- > 16 g / day ProGo™ treatment for 6 weeks showed a ~14% rise in hemoglobin levels versus WPI control
- > ProGo™ contains a low Iron content *per se* (3.1 mg/kg) so it is likely that bioactive peptides may be playing a role
- > Serum ferritin levels were elevated during treatment lending support to this conclusion

Journal of Nutrition & Food Sciences (2015) **5(4)** 379



* significantly different between protein drinks

Agenda

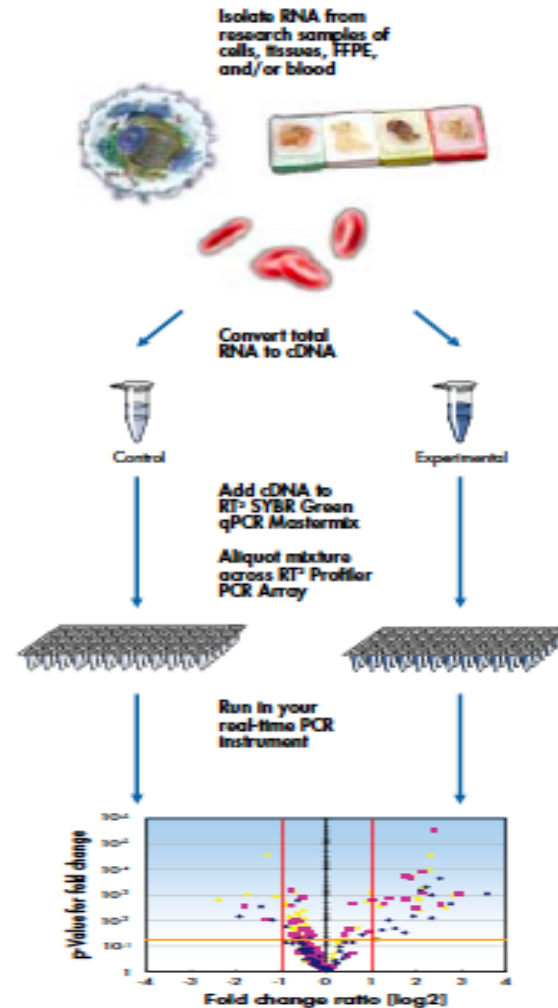
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ProGo 



ProGo[®] - oxidative stress-related genes regulation assay in HGEPP cells

- Human Gingival cell line reflects oral use of ProGo
- The Oxidative Stress RT² profiler PCR arrays (84 protective genes) from Qiagen N.V was used for the assay
- HGEPP cells (CellnTec AG) are incubated with ProGo solution (100uM) for 24 hrs at 37C
- RNA was isolated (DNA free), cDNA generated from it and treated with RT² SYBR[®] Green fluor qPCR mastermix.
- iCycler PCR system from Bio-Rad Inc., USA was used for the RT-PCR



RT² Profiler PCR Array plate layout

	1	2	3	4	5	6	7	8	9	10	11	12
A	01	02	03	04	05	06	07	08	09	10	11	12
B	13	14	15	16	17	18	19	20	21	22	23	24
C	25	26	27	28	29	30	31	32	33	34	35	36
D	37	38	39	40	41	42	43	44	45	46	47	48
E	49	50	51	52	53	54	55	56	57	58	59	60
F	61	62	63	64	65	66	67	68	69	70	71	72
G	73	74	75	76	77	78	79	80	81	82	83	84
H	HK1	HK2	HK3	HK4	HK5	GDC	RTC	RTC	RTC	PPC	PPC	PPC

Housekeeping genes Genomic DNA control Reverse transcription controls Positive PCR controls

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GenBank	Symbol	Description	Fold Change	P Value
NM_000041	APOE	Apolipoprotein E	2.76	0.028
NM_134268	CYGB	Cytoglobin	2.59	0.041
NM_000502	EPX	Eosinophil peroxidase	3.05	0.037
NM_002032	FTH1	Ferritin, h polypeptide 1	4.82	0.033
NM_001498	GCLC	Glutamine-cysteine ligase	4.91	0.016
NM_000581	GPX1	Glutathione peroxidase 1	2.88	0.034
NM_000178	GSS	Glutathione synthetase	2.63	0.011
NM_001513	GSTZ1	Glutathione transferase z1	2.49	0.045
NM_002133	HMOX1	Heme oxygenase (decyc) 1	5.63	0.017
NM_000250	MBL2	Mannose binding lectin 2	2.42	0.028
NM_012331	MSRA	Methionine sulfoxide red A	2.07	0.034
NM_000625	NOS2	Nitric oxide synthase 2	2.76	0.026
NM_000903	NQO1	NAD(P)H dehydro quin 1	3.85	0.019
NM_181652	PRDX5	Peroxiredoxin 5	2.74	0.039
NM_203472	SELS	Selenoprotein S	2.26	0.042
NM_000454	SOD1	Superoxide dismutase 1	3.50	0.013
NM_000697	ALOX12	Arachidonate 12-lipo	0.29	0.040
NM_001979	EPHX2	Epoxide hydrolase 2	0.38	0.022
NM_004528	MGST3	M glutathione S-tran 3	0.46	0.031
NM_000250	MPO	Myeloperoxidase	0.37	0.046
NM_000265	NCF1	Neutrophil cytosolic factor 1	0.43	0.015
NM_016931	NOX5	NADPH oxidase, Ca 5	0.41	0.023
NM_002574	PRDX1	Peroxiredoxin 1	0.40	0.034
NM_000963	PTGS2	Prostaglandin endop syn 2	0.35	0.027
NM_080725	SRXN1	Sulfiredoxin 1	0.42	0.038

ProGo[®] - FTH1, HMOX1, ALOX12 showed a dose dependent response

- > **FTH1 (encodes heavy subunit of ferritin)**
 - Sequestration of iron to prevent toxicity
 - Increased synthesis reduces the accumulation of ROS in response to an oxidant challenge.
 - Our results show that overexpression need not be only in response to an oxidant challenge and may be induced by specific gene activator compounds.
 - In our human study, ProGo was initially suspected to directly increase iron uptake in situationally anemic women
 - This gene assay result indicates an alternate mechanism for this by direct up-regulation of the FTH1 gene, increasing ferritin expression.

Genebank	Gene	Description	Fold Change		
			25	50	100 µM/ml
NM_002032	FTH1	Ferritin, heavy polypeptide 1	2.10	2.96	4.82
	P Value		0.036	0.045	0.033
NM_002133	HMOX1	Heme oxygenase (decyc) 1	1.92	3.47	5.63
	P Value		0.040	0.021	0.017
NM_000697	ALOX12	Arachidonate 12-lipoxygen	0.49	0.37	0.29
	P Value		0.065	0.062	0.040

ProGo[®] - FTH1, HMOX1, ALOX12 showed a dose dependent response

> **HMOX1**

- HMOX1 expression is induced by oxidative stress and confers cyto-protection.
- The HO1 (hemeoxygenase) enzyme catalyzes the degradation of heme, which produces biliverdin, ferrous iron, and carbon monoxide.
- Carbon monoxide released from heme oxygenase reactions has been shown to influence vascular tone and the function of nitric oxide synthase.
- The upregulation of HMOX1 could result in increased circulatory iron and hence ferritin but does not account for the increase in hemoglobin levels seen in our human trials

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ProGo[®] - FTH1, HMOX1, ALOX12 showed a dose dependent response

> **ALOX12**

- ALOX12 gene encodes a lipoxygenase enzyme that metabolizes AA into 15(S)-HETE, a hormone-like signaling agent, involved in inflammation response and metabolic syndrome.
- Elevated lipoxygenase has been implicated in type 1 diabetes, in the brown to white adipose tissue changes in obese individuals and in excessive production of ROS with inflammation.
- Down-regulation of ALOX12 and its metabolite(s) may contribute to the slower development and progression of obesity, diabetes, hypertension, and metabolic syndrome.

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ProGo 



*ProGo® - Directly Marketable
and A Rich Source of New Leads*

> **Active Lead Development**

- A dietary “low-iron sourced” product for iron deficiency anemia
- Anti-inflammatory dietary supplement for managing metabolic syndrome and pre-diabetic condition
- Enteral formulation for improving NEC in neonates

Weight Management

- Develop a series of products based on new formulations that can be used in healthy weight management
- Proven biological mode of action and effect for BMI control
- Dose regimen and formulation optimization : protein drinks, gels, and quick snacks

Cosmetics

- High Type I collagen content for the development of oral beauty products

Quick Uptake & Recovery Protein

- Indicative of good muscle recovery post exercise
- Increased energy and vitality

This is different™

> hofsethbiocare.com

