

## Effects of Biotin-rich Functional Food (Whalgichan) on Hair Growth and Biological Stimulation in Rat and Human

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### Abstract

For the development of functional food for hair-growth stimulation, this study has tried an *in vivo* and clinical test. As an *in vivo* test male Sprague Dawley rats and as a clinical test 27 baldheaded or loosehaired men were recruited. Before the experiment, the total hair count in 6 mm<sup>2</sup> of the designated area was 46.5. In four weeks and eight weeks of the functional food feeding it increased to 61.8 and 75.3, respectively. Hence the net increase was totaled at 62% in eight weeks. Also, depilation was decreased by 28%. Before the experiment, average hair loss was 65.7. In four weeks and eight weeks of the functional food feeding it decreased to 55.2 and 47.3, respectively. LDL and phospholipids were decreased by 42% and 36%, respectively during that period. However, HDL was increased by 21%. Forty percent of the subjects responded that itching of head skin was reduced and 34% responded to have reduced dandruff. No side-effects among the subjects were examined and no other blood parameters were significantly affected by the diet. The results in this study suggest that biotin-rich functional food may stimulate cholesterol and lipid metabolism and blood flow leading to the growth of new hair and prevention of hair loss.

**Key words:** alopecia, biotin, hair growth, biological stimulation

### INTRODUCTION

Alopecia has been constantly increasing recently among adults and the age of acquiring alopecia is shortened. Various hair loss prevention products have been produced but have only temporary effects or cause many side-effects. Topical ointments, in particular, revealed to cause itching of the head skin, excess production of dandruff and scalp fats and dermatitis.

It is known that the total number of human hair follicles is estimated at 80,000 to 100,000 covering the scalp and to have the life cycle of about 3 years. It is considered that alopecia has begun in the case of persistent falling out of more than 65~75 hair follicles per day. The basic hair follicle structure remains essentially the same through the range of mammalian species with modifications for specialized functions. The maintenance and formation of the hair follicle depend on the interaction between dermal and epidermal components. Dermal papilla (DP) is vital to the development of hair follicles and associated modified structures like sebaceous glands. The development of a DP marks the site for future development of a hair follicle.

Hair follicle cells can not actively differentiate without the supply of sufficient nutrients. Biotin, in particular, plays an indispensable role in the growth of new hair follicles. Alopecia, ataxia, skin rash and developmental delay were commonly found in children and adults who were deficient in biotin and biotin enzyme (1-7). Biotin acts as a coenzyme for many es-

sential biological processes in the human body including gluconeogenesis, fatty acid synthesis and the metabolism of amino acids (8-17).

Lack of nutrients for hair follicles shortens the period of hair-life as well. Under this circumstance, the hair stem becomes thinner and weaker and eventually stops its growth. As a result, depilation will continually proceed. Intangible factors such as heredity, pollution, use of anticancer drugs, hypertemperature, disease and stress may also have effects on hair loss.

Though the efficacy is not clearly established, one of the most common hair loss prevention agents on the market, minoxidil (18), a piperidino pyrimidine derivative, was initially developed for the purpose of curing hypertension by way of extending the blood vessel, which implies that peripheral blood circulation and nutrient supply is vital to the prevention of hair loss. This study has examined the clinical efficacy of biotin-rich functional foods for the prevention of hair loss and further for the growth of new hair.

### MATERIALS AND METHODS

#### Animals and experimental design

Male Sprague Dawley rats purchased from Korea Experimental Animal Center weighing approximately 80 g were randomly divided and were maintained in a 12:12 dark and light cycled room. Before the start of the experiment animals were fed chow diet for a week and then divided into four groups:

the control (C), high fat (CL), active ingredient (CW) and high fat and active ingredient (CLW) groups. Diet groups and the experimental diet composition is shown in Table 1.

#### Experimental diet composition

Animals were allowed to have free access to diet (*ad libitum*) and were provided a powdered mixed diet based on the AIN-76 formula. The rat diet was composed of casein (14%), corn oil (4%), corn starch (20%), DL-methionine (0.18%), cellulose (2%), Vitamin mix (1%), Mineral mix (3.5%) and sucrose to 100%. The high fat diet contained 0.25% of sodium colate, 10% of lard and 1% of cholesterol. The materials for the active ingredients were obtained locally in Kangwon province, washed and ground. The active ingredients were composed of black sesame 15%, black bean 35%, black rice 5%, Job's tears 5%, pine nut 5%, perilla japonica 4%, seaweeds 20%, brewer's yeast 8% and wild herb extract 3% on a wet weight basis. Nutrition analysis per 100 g of the active ingredient showed 475.1 mg of Ca, 8.1 mg of thiamin, 3.3 mg of riboflavin, 15.1 mg of ascorbate, 145 µg of biotin and 3.2 mg of zinc. Active ingredients were supplied at 10% of the total diet.

#### Preparation of tissue specimen for assay

Animals were fasted for 12 hours and weighed before sacrificed by ether anesthesia and cervical dislocation. Blood was taken by cardiac puncture, organs including liver and kidney were taken, weighed and rinsed by physiological saline. Liver sample of 1 g was homogenized with a 0.25 M sucrose buffer and centrifuged at 600 g (Microspin 24S, Sorvall instruments, USA) for 15 minutes, pellets were discarded and supernatants were obtained and kept frozen at -70°C for enzyme assay.

#### Analysis of serum lipid components

Serum triglycerides and cholesterol were assayed at 555 nm for 5 minutes, 37°C with a Johnson and Johnson Ektachem spectrophotometer (USA). Slide kits (Johnson and Johnson, USA) of different kinds were used for each lipid component analysis. HDL-cholesterol, LDL-cholesterol and phospholipid were analyzed at 505 nm with a Beckman spectrophotometer (DU series-70, USA) using kit (Wako Co., Japan).

#### Analysis of liver function-index enzymes

The enzymatic activity of glutamic oxaloacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) was measured at 340 nm, 37°C for 5 minutes and 3 minutes, respectively with Johnson and Johnson spectrophotometer (USA)

Table 1. Experimental design of animals

Group	Diet
C	Basal diet
CW	Basal diet + Active ingredient <sup>1)</sup>
CL	Basal diet + High fat <sup>2)</sup>
CLW	Basal diet + High fat + Active ingredient

<sup>1)</sup>Active ingredients: Refer to experimental diet composition

<sup>2)</sup>High fat: 10% of lard, 1% of cholesterol and 0.25 % of sodium colate in the diet.

using kits.

#### Glucose tolerance test

Rats were secured in a plastic animal holder and rested for 10 minutes. Sucrose of 7.5 g/kg, body WT was orally administered through a stomach tube. Blood was drawn from the tail vein at 0,30, 60, 90 and 120 minutes of oral glucose intake. Blood glucose was measured instantly with glucometer (National, Model EW272W, Japan).

#### Human subjects

The recruitment of volunteers for human study was advertised in the Kangwon Daily newspaper during October 1998. Forty alopecia subjects were selected out of 100 volunteers. The results from 27 subjects were reflected in the final data excluding 13 dropouts during the 8-week-experimental period. Subjects were instructed to take 4 g per pack of active ingredients 3 times a day together with regular meals.

#### Measurement of growth and loss of hair

The total number of hairs in a 6 mm<sup>2</sup> area was measured before and after 4 and 8 weeks of the active ingredient intake. In order to minimize experimental error, two fixed spots were chosen and the hair-counts were averaged. Subjects were instructed to collect and count the total lost hair during hair washing and shower. Hair washing intervals were scheduled and selected by the subjects. Lost hair was counted twice and averaged.

#### Anthropometric measurement and blood analysis

Human body composition and fat analysis was performed using a body composition analyzer (Biodynamics, Model 310, USA) with a pair of sensor pads attached to the right wrist and ankle joint while the other pair of cable pads were attached to the right hand and foot with a relaxed body position. Changes of body weight and height before and after taking the diet were analyzed. Blood glucose was measured with a "Surestep" glucometer (LIFESCAN Co., USA) right after blood was drawn and the blood pressure with an "EW272w" (National, Japan) at the designated time to reduce error from daily fluctuation. Blood composition was analyzed with a Berlinger Menheim autoanalyzer (West Germany).

#### Statistical analysis

The data from individual experiments was expressed as the mean ± standard error of means (SEM). Statistical analysis was performed by SAS (statistical analysis system) program and Duncan's multiple range test at  $p < 0.05$ .

## RESULTS

#### Diet consumption and weight gain of the rat

Diet consumption of the control group (C) was decreased, while that of high fat groups was increased. At the same time, the supplement of active ingredients for six weeks tended to increase the body weight of the experimental rat (Table

2). The liver weight of the rat was increased by the supplement of active ingredients and high fat diet (Table 3). However, the kidney and spleen weight did not change significantly.

#### Rat glucose tolerance test

The blood glucose level was increased by high fat and active ingredients (Table 4). The peak blood glucose was achieved at 60 minutes of oral glucose administration and thereafter it decreased slowly. Even at 120 minutes, the blood glucose level did not reach the fasting level and remained high.

**Table 2.** Body weight gain and food intake of experimental rats

Period	Group	Body weight gain	Food intake (g/day)
3 wks	C <sup>1)</sup>	55.1 ± 2.51 <sup>2)</sup>	25.9 ± 2.42
	CW	68.7 ± 7.26	31.2 ± 0.96
	CL	72.6 ± 3.21	37.5 ± 1.75
	CLW	86.8 ± 4.33	28.7 ± 1.29
6 wks	C	111.5 ± 5.72	15.8 ± 0.96
	CW	146.5 ± 4.58	20.7 ± 1.24
	CL	145.5 ± 3.79	22.2 ± 2.34
	CLW	156.3 ± 6.67	25.3 ± 1.85

<sup>1)</sup>C: Control

CW: Active ingredient group

CL: High fat group

CLW: High fat and active ingredient group

<sup>2)</sup>Mean ± S.E.M (standard error of mean)

**Table 3.** Organ weights of rat

Period	Group	Organ weight (g)		
		Liver	Kidney	Spleen
3 wks	C <sup>1)</sup>	4.570 ± 0.831 <sup>2)</sup>	1.063 ± 0.093	0.413 ± 0.084
	CW	5.088 ± 0.334	1.170 ± 0.062	0.473 ± 0.027
	CL	4.640 ± 0.507	1.140 ± 0.086	0.403 ± 0.064
	CLW	4.618 ± 0.393	4.077 ± 3.012	0.418 ± 0.048
6 wks	C	5.995 ± 0.945 <sup>c3)</sup>	1.468 ± 0.203	0.600 ± 0.114
	CW	11.030 ± 0.808 <sup>a</sup>	1.798 ± 0.067	0.498 ± 0.045
	CL	9.118 ± 0.933 <sup>ab</sup>	1.598 ± 0.133	0.573 ± 0.038
	CLW	7.348 ± 0.281 <sup>bc</sup>	1.683 ± 0.083	0.545 ± 0.045

<sup>1)</sup>Refer to Table 2.

<sup>2)</sup>Mean ± S.E.M (standard error of mean)

<sup>3)</sup>Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test at p < 0.01.

Values with no statistical significance among groups were not indicated.

**Table 4.** Glucose tolerance test of rats

Group	Fasting	30 min	60 min	90 min	120 min
C <sup>1)</sup>	61.5 ± 9.5 <sup>2)3)</sup>	427.5 ± 18.5	862.5 ± 29.5	523.5 ± 46.5	381.0 ± 94.0
CW	78.5 ± 4.5 <sup>ab</sup>	465.0 ± 16.5	758.0 ± 75.0	587.5 ± 7.5	405.0 ± 75.0
CL	97.5 ± 3.5 <sup>a</sup>	397.5 ± 7.5	727.5 ± 22.5	525.6 ± 94.4	394.6 ± 95.5
CLW	74.0 ± 2.0 <sup>b</sup>	512.5 ± 27.5	920.0 ± 15.0	612.0 ± 75.0	422.5 ± 82.5

<sup>1)</sup>Refer to Table 2.

<sup>2)</sup>Mean ± S.E.M (standard error of mean)

<sup>3)</sup>Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test at p < 0.05.

Values with no statistical significance among groups were not indicated.

#### Increase of human hair growth and reduced hair loss

Before the experiment, the total hairs counted in 6 mm<sup>2</sup> of the designated area was 46.5 (Table 5). In four weeks and eight weeks of the functional food feeding it increased to 61.8 and 75.3, respectively. Hence the net increase totaled 62% in eight weeks (Fig. 1).

Also, depilation was decreased by 28%. Before the experiment, average hair loss was 65.7. In four weeks and eight weeks of the functional food feeding it decreased to 55.2 and 47.3, respectively.

#### Changes of human serum lipid parameters

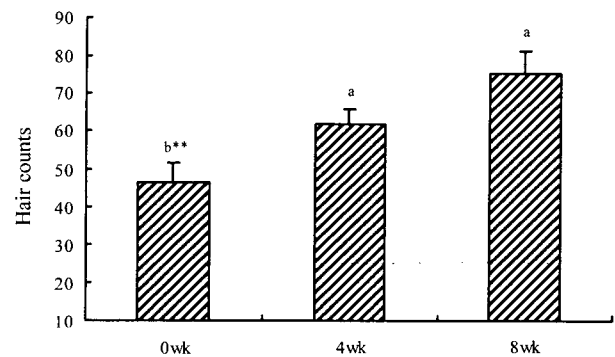
The intake of the active ingredients caused a decrease of triglycerides and cholesterol (Table 6). LDL, in particular, decreased to 10.78 mg/dl and 9.51 in 4 weeks and 8 weeks, respectively from 16.46 mg/dl at the start of the experiment, which totals a 42% decrease. On the other hand, HDL was increased from 33.09 mg/dl of the baseline to 39.50

**Table 5.** Number of human loose hairs counted

Period of intake	Number of loose hairs counted
0 wk	65.646 ± 7.794 <sup>1,2)</sup>
4 wk	55.167 ± 7.856
8 wk	47.286 ± 6.161

<sup>1)</sup>Mean ± S.E.M (standard error of mean)

<sup>2)</sup>Values with no statistical significance among groups were not indicated.



**Fig. 1.** Effects of active dietary ingredients on the total number of human hair counts in 6 mm<sup>2</sup> of the designated area. Bar and error bar represent mean ± standard error of mean. Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test at p < 0.01.

**Table 6.** Changes of human serum lipid parameters

Period of intake	Triglyceride (mg/dl)	Cholesterol (mg/dl)	LDL-cholesterol (mg/dl)	HDL-cholesterol (mg/dl)	Phospholipids (mg/dl)
0 wk	178.92 ± 16.73 <sup>1)</sup>	193.86 ± 7.38 <sup>2)</sup>	16.46 ± 1.87 <sup>a</sup>	33.09 ± 1.47	16.46 ± 1.87 <sup>a</sup>
4 wk	170.39 ± 12.29	170.31 ± 7.75 <sup>b</sup>	10.78 ± 0.63 <sup>b</sup>	39.50 ± 2.76	10.78 ± 0.63 <sup>b</sup>
8 wk	180.69 ± 17.88	197.39 ± 7.08 <sup>a</sup>	9.51 ± 0.92 <sup>b</sup>	40.11 ± 9.99	10.51 ± 0.92 <sup>b</sup>

<sup>1)</sup>Mean ± S.E.M (standard error of mean)

<sup>2)</sup>Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test at  $p < 0.01$ .

Values with no statistical significance among groups were not indicated.

mg/dl in four weeks and 40.11 mg/dl in eight weeks representing 19% and 21% of the increase. Phospholipids were decreased by 36% in eight weeks from the baseline of 16.46 mg/dl to 10.51 mg/dl.

#### Changes of human liver-function index enzymes

Liver function index enzymes of GOT and GPT were not influenced by the diet (Table 7). GOT activity was increased from 17.462 to 22.143 units in eight weeks. GPT activity was decreased from 18.88 units to 14.68 units and then increased to 27.704 units.

#### Changes of human body composition, lipids and BMR

The overall body fat tended to decrease from 20.619% of the baseline to 19.465% in four weeks and 19.707% in eight weeks (Table 8). The actual body fat weight from the baseline of 14.881 kg was decreased to 14.013 kg in four weeks and 14.152 kg in eight weeks, indicating that lipid metabolism is stimulated by the active diet ingredient. Lean body weight instead was slightly increased. BMR also was increased by the diet containing various sea weeds, which may have stimulated the synthesis of thyroxine.

#### Other biological changes

The WBC, RBC, HCT and other blood components were not influenced by the diet. Lymphocytes, monocytes and other

immune factors were not significantly changed. The itching of head skin and dandruff production was decreased and the overall head skin condition was improved. Constipation also was improved and vitality was recorded to be strengthened by the survey. And no side effects following the intake were observed.

## DISCUSSION

Male pattern alopecia occurs in almost half of the adult men and increases with aging. Alopecia is particularly common among adults with hypercholesterolemia. Biotin deficiency causes irregularities of fat metabolism including fatty liver and elevated palmitoleic acid concentration in the liver and cholesterol in the serum (19-24). The deficiency of biotin and biotinase were caused by alterations in brain content of saturated fatty acids or cholesterol. The deficiency produced a central nervous system dysfunction in rat brain tissue and an abnormal composition of serum and hepatic saturated fatty acids (25-28).

In this study the biotin-rich ingredients significantly lowered serum LDL and triglyceride and increased HDL. At the same time, the diet stimulated 62% of new hair growth, suggesting that the increased fat metabolism might facilitate the synthesis of new hair follicles. Abnormal fatty acid metabolism of men with baldness was associated with carboxylase, a biotin enzyme deficiency (29,30). Biotin functions as an essential cofactor for the catalase enzyme, which catalyzes a critical step in the intermediary metabolism of humans and animals (21,31).

High blood pressure and cardiovascular diseases caused by long-term intake of high fat, cholesterol and excess calories can possibly be reversed by the improvement of the blood constituents of the human body. The results from the topical application of 2.5% pentadecanoic glyceride (PDG) showed only a 7.18% and a 10.79% increase in 12 and 24 weeks,

**Table 7.** Changes of human liver function enzyme activities

Period of intake	Glutamic oxaloacetic transaminase (U/L)	Glutamic pyruvic transaminase (U/L)
0 wk	27.462 ± 2.069 <sup>1)2)</sup>	18.880 ± 1.529
4 wk	19.308 ± 1.951 <sup>c</sup>	14.680 ± 1.066
8 wk	22.143 ± 1.784 <sup>a</sup>	17.704 ± 1.597

<sup>1)</sup>Mean ± S.E.M (standard error of mean)

<sup>2)</sup>Values within the same column with different alphabets are significantly different among groups by Duncan's multiple range test at  $p < 0.05$ .

**Table 8.** Changes of human body compositions

Period of intake	Percent body fat (%)	Body fat weight (lbs)	Bioresistance (ohms)	Total body water (L)	Lean body weight (lbs)	BMR (cal/day)
0 wk	20.619 ± 0.902 <sup>1,2)</sup>	14.881 ± 0.859	469.929 ± 9.558	41.246 ± 1.055	57.252 ± 1.487	1740.259 ± 45.175
4 wk	19.465 ± 1.194	14.013 ± 1.068	469.208 ± 13.419	40.792 ± 1.404	57.397 ± 1.838	1742.957 ± 64.441
8 wk	19.707 ± 1.066	14.152 ± 0.887	461.704 ± 13.506	42.056 ± 1.516	58.015 ± 1.694	1763.556 ± 51.449

<sup>1)</sup>Mean ± S.E.M (standard error of mean)

<sup>2)</sup>Values with no statistical significance among groups were not indicated.

respectively from photographic hair counts (32). And the use of photographic hair counts, objective examination and other quantitative methods (33,34) have been in dispute for the consistency of the outcome. Hair growth with the dietary supplement in this study was increased by 62% for 8 weeks compared to a 7.18% increase for 12 weeks of the study (32) done locally. Along with the outcome of a 30% increase of hair loss prevention, the biotin-rich diet supplementation brought a significant stimulation of the growth and maintenance of hair. A nonionic detergent polysorbate 60 turned out to be of no significant effect on male pattern alopecia (35) at the trial for 141 subjects for 16 weeks.

A recent study dealing with the relationship between male pattern alopecia and coronary heart disease (CHD) after 11 years of follow-up of 22,071 US male physicians has shown that alopecia, particularly vertex baldness, was strongly associated with CHD risk resulting from hypertension or high cholesterol levels (36). In fact a 3-fold higher risk of myocardial infarction was observed in men with vertex baldness comparing the normal. Men with vertex baldness, hypertension and hyperlipidemia showed even higher risks of myocardial infarction before the age of 60 (37-41), indicating the existence of a link between lipid metabolism and alopecia. On the other hand, women with cystic ovary syndrome and elevated androgen levels were suggested to develop atherosclerosis (42) and high levels of triglycerides and low levels of HDL cholesterol (43).

Our findings in this study confirm that increased HDL and decreased LDL cholesterol and triglycerides for men with alopecia stimulate new hair growth and prevent hair loss. Although it may require more time than medical therapy, the functional food developed (Whalgichan) can be assured as the safe way for the regeneration of lost hair as well as for the growth of new hair. Other senescence diseases can also be improved by the intake of functional food with a relatively long-term therapy plan partly because it provides good quality protein, essential fatty acids, fiber, high calcium and minerals and other nutrients from natural ingredients.

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