Pilot study for an age- and gender-based nutrient signaling system for weight control

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Abstract Nutrient signaling has recently shown how nutraceuticals regulate specific functions of the brain and adipose tissue. In this pilot study to find an effective nutrient signaling system to cause weight loss, a double-blind placebo-controlled trial using leucine, olive oil, and fish oil was conducted on volunteers to signal metabolic and appetite effects to regulate body weight, while controls took only fiber. Men and women aged 18–26 and 39–62 years were given different dosages that they took orally twice daily for 14 days while recording body weight, followed by 2 weeks to check rebound. Most young men and women lost weight on low dose leucine and olive oil. Mature men required higher doses. Mature women's weight was affected least, though results are consistent with a hypothesis that sufficient leucine and docosahexaenoic acid would be effective. Determining how age affects signaling pathways by nutrients will be important to reduce risk of chronic disease associated with age and obesity. This pilot study has led to hypotheses of practical strategies.

Keywords Leucine · Fish oil · Oleic acid · Branched chain amino acids · Docosahexaenoic acid · DHA · Olive oil · mTOR · Obesity · Weight loss

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Abbreviations

BCAA	Branched chain amino acids
Cntl	Control
DHA	Docosahexaenoic acid
EPA	Eicosapentaenoic acid
Fsh	Fish oil
Hi	High dose (12 g/day in divided doses)
Lo	Low dose (6 g/day in divided doses)
Leu	Leucine
M	Men
mTOR	Mammalian Target of Rapamycin
O	Older
Olv	Olive oil
W	Women
Y	Younger

Introduction

Obesity is a major factor increasing the risk of chronic disease with age (WHO 2004; Woo et al. 2001). The rate of obesity has increased significantly worldwide (Bray 2004). In well-controlled longterm studies, people on many popular diet plans fail to lose weight (Zivkovic et al. 2007).

In the past decade, biological signaling pathways have been recognized to control many metabolic processes (Bhalla and Iyengar 1999). Studies began to recognize that beyond providing nutrition, certain molecules in food activate signaling pathways rele-

Table 1 Study considerations. The following variables were evaluated in this study, using abbreviations shown

Category	Symbol	Details	
Dosage	Lo	Low	6 g/day
	Hi	High	12 g/day
Branched chain mino acid	Leu	Leucine	
Fatty acid	Olv	Olive oil	
	Fsh	Fish oil	
Control	Cntl	Cellulose	
Gender	M	Men	
	W	Women	
Age	O	Older	39–62 years
	Y	Younger	18–24 years

vant to weight control. In 2006, three studies indicated how nutrients may signal metabolic controls related to body weight.

The first study was done with leucine. Other studies (She et al. 2007; Eley et al. 2007) have shown that branched chain amino acids (BCAA) produce anabolic responses to build muscle. But BCAA also stimulate the nutrient signaling pathway known as the mammalian Target of Rapamycin (mTOR) pathway (Sabatini et al. 1994). The protein regulated by the mTOR pathway is a serine-threonine kinase that regulates cell-cycle progression and growth by sensing changes in energy status. Cota et al. (2006) described the complex effects of mTOR signaling and then demonstrated in rats that leucine produces two signals in the brain in specific regions of the hypothalamus and the arcuate nucleus. They also demonstrated that those two signals decreased both food intake and body weight.

The second study was done with oleic acid. A signaling system proposed by Hsu and Huang (2006) could regulate metabolism in adipose tissue in rats. They proposed two signals that would occur in fat cells in response to oleic acid. Fatty acid catabolic genes would be upregulated while lipid storage genes would be downregulated.

The third signaling system was found by Kim et al. (2006) using docosahexaenoic acid (DHA). This fatty acid commonly associated with fish oil was shown to produce two important signals, one inhibiting adipocyte differentiation and another causing apoptosis in preadipocytes.

The goal of this pilot study was to begin exploring which different combinations and dosages of branched chain amino acids and fatty acids (Table 1) might affect weight loss in humans as a function of age and gender. Given the often contradictory reports from obesity studies, specific hypotheses were set forth based on familiarity with nutritional literature and the above citations. Although the hypotheses may be controversial, clearly stated hypotheses invite direct experimental exploration.

Hypotheses

1. Eating the combination of leucine and a fatty acid will cause weight loss, because the signals will work synergistically.
2. Eating twice the dosage of leucine and a fatty acid will make weight loss more likely because stronger signals will be delivered. The initial doses selected for this study were extrapolated from the rat studies.
3. Fish oil is more likely to cause weight loss than olive oil, because DHA is essential in the diet while oleic acid is not.

Table 2 Characteristics of participants

No.	Age (years)		Weight (kg)		Dosages (g/day)			
Group	Max	Min	Min	Max	Leu	Olv	Fsh	Cntl
Women over 38 years								
7	58	44	58.5	114.3	12	12	0	0
5	52	40	68.0	99.3	12	0	12	0
5	59	39	56.7	125.6	6	6	0	0
4	58	47	70.3	100.7	0	0	0	12
Women 18–26 years								
10	23	18	54.9	85.7	12	12	0	0
1	21	n/a	63.0	n/a	12	6	6	0
2	23	22	62.6	66.2	12	0	12	0
3	20	18	71.2	78.5	6	6	0	0
2	24	19	69.4	77.1	0	0	0	12
Men over 38 years								
2	53	45	127.0	128.8	12	6	6	0
2	62	58	77.1	106.6	6	6	0	0
4	62	41	95.3	117.5	0	0	0	12
Men 18–26 years								
4	22	19	96.2	138.8	12	6	6	0
4	23	18	62.6	97.5	6	6	0	0
4	26	24	73.9	92.1	0	0	0	12

Fifteen groups took different dosages of leucine, olive oil, fish oil, and cellulose when waking in the morning and again 0–3 h before the evening meal

Table 3 Participant dairies

Record Type	Number
Signed informed consents	72
Assigned to receive tablets	68
Participants providing records	60
14-day weight record	59
2-week rebound record	48
Diary comments received	31
Comment not tablet related	13

Public advertising invited college-related audiences to consider participation; responses after public announcements of the trial are shown

4. It will be harder to cause weight loss in older (O) than younger (Y) people because signaling pathways may be less responsive.
5. It will be harder to cause weight loss in women (W) than in men (M) because women have evolved to survive to care for offspring.

These hypotheses resulted in the design of this study. I extrapolated combinations and dosages of nutrients from in vitro and rodent studies. The purpose of the study was to identify specific protocols that might have an effect in a trial large enough to achieve statistical significance. This pilot study provided an efficient way to determine protocols most likely to succeed in producing significant weight loss.

Materials and methods

Study population

The Beloit College Institutional Review Board approved the trial. Of 68 healthy volunteers who provided informed consent after the procedure had been fully explained, 8 withdrew without reason and 1 developed an illness unrelated to the study. Characteristics of the 59 who actively participated in the study are shown in Table 2.

Regimen and compliance

All signaling molecules were provided in 1-g capsules. Half of the daily dosage was taken upon waking in the morning, 0 to 3 h before breakfast. People then weighed themselves before eating. The second half of the pills was taken 0–3 h before the evening meal. Experimental groups swallowed high (12 g/day) or low (6 g/day) dosages of the BCAA leucine. They also took 6–12 g/day of either fish oil containing 0.12 g DHA and 0.18 g EPA/g fish oil, and/or olive oil containing 0.85 g oleic acid/g olive oil. Controls took cellulose tablets. Participants did not know which tablets they were taking. They took tablets for 14 days. They were encouraged to maintain normal behavior, not modifying eating or exercise habits during the trial. After 2 weeks of taking tablets, they recorded their weights 1 and 2 weeks later so that rebound effects could be evaluated.

Table 4 Possible side effects

Sex	Age (years)	Dosage	No. in group	No. diaries received	Comments
F	Over 38	Control	4	1	Trouble sleeping
F	Over 38	L1O1	5	1	Decreased appetite
F	Over 38	L2F2	5	2	Reduced appetite 1 day; feel bloated
F	Over 38	L2O2	7	4	Excess elimination; painful gas and bloating; swollen hands/joints day; flu last 4 days of rebound
M	Over 38	Control	4	1	No appetite 1 day
M	Over 38	L1O1	2	2	More energy; joints hurt 1 day
M	Over 38	L2O1F1	2	1	Constipated
F	18–26	Control	2	1	Nauseous 1 day
F	18–26	L2O2	9	2	Hungrier; feel great, more energy
M	18–26	Control	4	2	Hungrier; less tired
M	18–26	L2O1F1	4	1	Eating healthier foods

Of 59 people taking tablets for 14 days, 18 made comments that may be related. No comment came from more than 1 person in a group

Table 5 Weight change and rebound

No. in group	Sex	Dosages[a] Leu	Olv	Fsh	Cntl	Lgst 2 week loss	Avg 2 week loss	Smlst 2 week loss	% Loss	% N.C.	% Gain	Lgst 2 week rebnd	Avg 2 week rebnd	Smlst 2 week rebnd	% Loss	% N.C.	% Gain
Young women																	
1	F	2	1	1	0	−0.9	−0.9	n.d.	100%			0.9	0.9	n.d.	100		
3	F	1	1	0	0	−1.8	−1.0	0.0	67%	33%		−0.8	−0.4	0.0		67%	33%
10	F	2	2	0	0	−3.2	−0.6	1.4	60%	10%	30%	−1.8	−0.4	0.5	20%	40%	40%
2	F	2	0	2	0	−0.5	−0.2	0.0	50%	50%		n.d.	−0.2	n.d.		100	
2	F	0	0	0	2	−0.5	−0.2	0.0	50%	50%		−0.2	−0.2	n.d.		50%	50%
Women over 38																	
5	F	2	0	2	0	−1.1	0.1	1.6	60%		40%	−1.4	−0.2	0.2	20%	40%	40%
7	F	2	2	0	0	−1.8	−0.4	0.5	43%	14%	43%	−1.8	−0.5	0.7	57%	14%	28%
5	F	1	1	0	0	−1.3	−0.2	0.9	40%	20%	40%	−1.8	−0.4	0.6	60%	20%	20%
4	F	0	0	0	2	−0.9	0.0	1.4	50%	25%	25%	0.0	0.2	0.9		75%	25%
Young men																	
4	M	2	1	1	0	−3.2	−0.9	1.3	75%		25%	−2.7	−0.8	0.5	25%	50%	25%
4	M	1	1	0	0	−1.6	−0.2	0.9	25%	50%	25%	0.0	0.0	n.d.			100%
4	M	0	0	0	2	−1.8	−1.0	−0.5	100%			−0.9	0.2	1.8	25%	25%	50%
Men over 38																	
2	M	2	1	1	0	−3.6	−2.0	−0.5	100%			−0.9	−0.5	0.0		50%	50%
2	M	1	1	0	0	−0.7	−0.7	−0.7	100%			−3.6	−1.9	−0.1			100%
4	M	0	0	0	2	−2.3	−0.3	0.9	25%	50%	25%	0.9	1.7	2.5	50%	50%	

[a] 1 = Lo Dose, 2 = Hi Dose

Lgst Largest, *Avg* average, *Smlst* smallest, *Leu* leucine, *Olv* olive oil, *Fsh* fish oil, *Cntl* cellulose, *n.d.* no data received, *N.C.* no change, *rebnd* rebound

The maximum, minimum and average individual weight changes are shown for each group after 2 weeks taking tablets, and rebound 2 weeks after the last tablets were taken. Total daily dosage is shown. Weight loss (kg) is shown as a negative number

Fig. 1 Influence of leucine dose on weight loss. Weight loss by all participants was averaged based on dosage of leucine taken
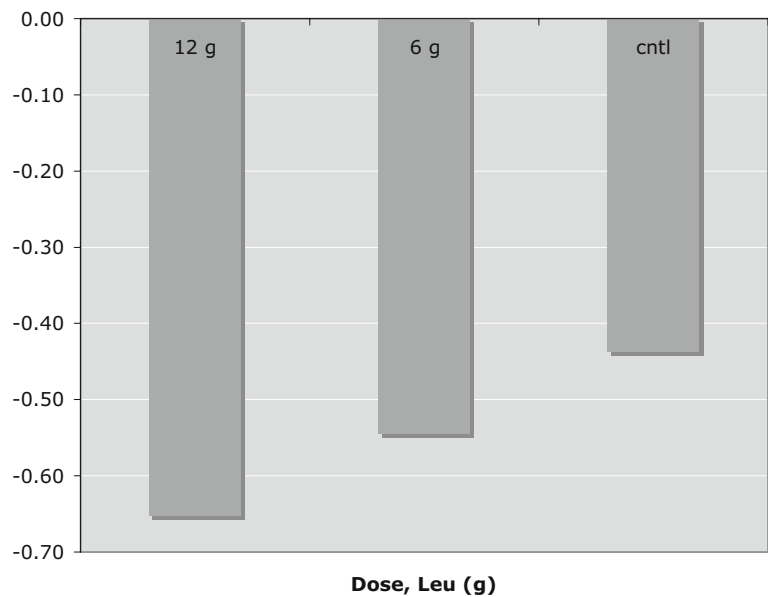
Participants recorded the times they took the tablets and recorded their weight each day. They were also asked to maintain a diary of possibly relevant events or feelings. All participants had email addresses and received daily messages about the importance of the accuracy of their record keeping. Because of health considerations (Partnership For Healthy Weight Management 1999) in the IRB proposal, people were instructed not to take tablets on days in which their weight had dropped more than 0.91 kg (2 lb)/week.

Endpoints

Weight loss was measured as the difference between weight the first morning and the last morning after taking tablets. Rebound was taken as the difference

between the last weighing after taking tablets and the weight 2 weeks later. To account for behavioral effects, participants were aware that this was a weight loss study where eating and exercise patterns were not to be modified, but did not know about the study design relating to age, gender, experimental, and control groups, or what the various tablets contained.

Statistics

Given safety considerations on weight loss and availability of resources, this pilot study was designed realizing there are more variables than were likely to be analyzed satisfactorily given the sample sizes in each cell. Application of analysis of variance (ANOVA) using the statistical software JMP did not

Fig. 2 Influence of fish and olive oil on weight loss. All dosages included 12 g leucine/day. Endpoint *S1* is weight loss, *S2* is rebound; *olv* olive oil, *fsh* fish oil, Total daily divided doses are: *Hi* = 12 g/day, *Lo* = 6 g/day
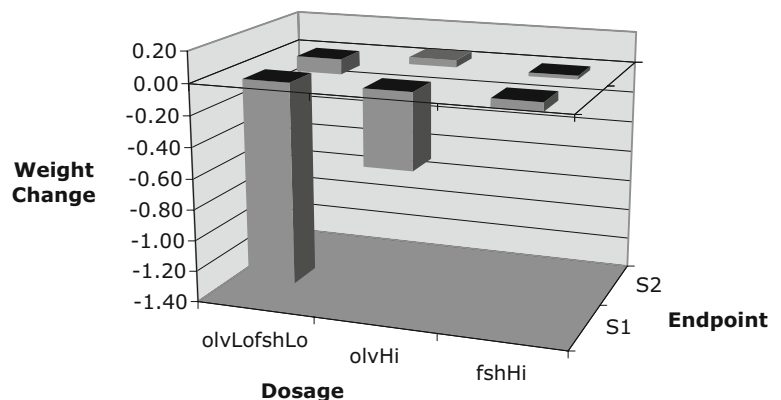
Table 6 Possible weight signals by nutrients

Nutrient example	Nutrient class	Signal 1	Signal 2	Reference
Leucine	Branched chain amino acid	To the brain to decrease appetite	To the brain to increase metabolic rate	Sabatini et al. 1994
Oleic Acid	Polyunsaturated fatty acids	To adipose tissue to upregulate fat catabolism	To adipose tissue to downregulate lipid storage gene expression	Hsu and Huang 2006
DHA	Omega-3 fatty acids	To adipocytes to inhibit differentiation	To pre-adipocytes to stimulate apoptosis	Kim et al. 2006

suggest any differences among treatments significant at the 5% level. Statistical significance would have been obtained if every subject taking both leucine and a fatty acid had lost the maximum weight permitted (0.91 kg) and every control had lost no weight. The truncation of weight loss at 0.91 kg/week, reached by some treatment subjects, results in underestimation of average effect of the treatments.

Results

Public advertising through Beloit College networks was used to recruit volunteers. Table 3 shows the responses received that led to 59 weight records analyzed for this trial. During instruction of volunteers including informed consent, many withdrew once they understood clearly what a blind controlled study involved. The 59 people finishing the trial remembered to record 97.5% of 826 daily weights. Complete rebound data was not provided by 12 of 59 who turned in 14-day weighing records.

Design of this pilot study has more effects than can be analyzed satisfactorily with available sample sizes. An analysis with "best possible" outcome did show that significance would have been possible with maximum weight loss difference from one group to another. The short duration does not allow greater weight loss that would be possible with a longer trial. Analysis of the values from weight records submitted by the participants is consistent with their accurately recording their weights.

Participants were provided daily diaries to record any comments in response to their experience. Table 4 shows the nature of those comments. All participants were encouraged to withdraw if their response to the tablets caused health concerns. No one gave any response on withdrawing from the study except the

person who reported being diagnosed with mononucleosis on the second day of taking tablets. Females over 38 provided the highest level of comments, but none withdrew.

This study was designed based on available resources to explore the five hypotheses presented in the introduction. The 59 participants were divided into 15 groups based on age, gender, and dosages. Table 5 summarizes weight change from first to last day of taking tablets, and weight regain (rebound) during the next two weeks. Values with negative signs indicate weight loss.

Discussion

Although knowledge related to nutrition and aging is changing rapidly, "the complex interrelationship between energy intake, energy expenditure and specific physical activity requirements for current humans remains very similar to that originally selected for Stone Age men and women who lived by gathering and hunting" (Cordain et al. 1998). The

Table 7 Results of mature women on 12 g leucine and 12 g olive oil

Year of birth	Initial weight (kg)	2 week loss (kg)	2 week rebd (kg)
1966	90.7	−1.8	0.5
1956	70.3	−0.9	−1.4
1964	58.5	−0.9	0.7
1948	114.3	0.0	0.0
1953	94.8	0.2	−0.7
1962	59.4	0.2	−0.5
1963	94.8	0.5	−1.8

Although there was an average loss of weight during tablets and rebound, the leuHiolvHi signals were not sufficient to cause weight loss in all mature women

results of this study may provide further insight into how to reveal those relationships and what they may be. The design of this study also demonstrates the value of double-blind design in weight loss studies where placebo effects can be substantial. The study tested the five hypotheses in the Introduction.

The average weight loss results were consistent with hypotheses 1 and 2, although there were individuals who did not lose weight. Eating the combination of leucine and a fatty acid did result in weight loss, and eating twice the dosage of leucine and a fatty acid resulted in more weight loss, as shown in Fig. 1.

Resources for the study allowed initial exploration of hypothesis 3. Fish oil tablets contained only 12% DHA, while the olive oil contained 85% oleic acid. A comparison of those taking 6 g olive oil (5.1 g oleic) and 6 g fish oil (0.72 g DHA) versus 12 g olive oil is shown in Fig. 2. These results reflect that the combination of the two fatty acids may have a greater effect than either alone. Based on these results and reports mentioned in the Introduction, the signaling systems of DHA and oleic acid indicated in Table 6 may be different and interact synergistically.

Hypotheses 4 and 5 are consistent with our results, as mature women lost the least weight for every dosage for which they were tested. Women may have evolved to retain body weight after maturity even under conditions of scarcity (Cordain et al. 2005). A systematic review of weight loss studies did not find gender to be a significant factor in the type of fat that was lost during dieting (Chaston and Dixon 2008), while a study of weight loss after bariatric surgery found more weight loss in females (Melton et al. 2008), so hypothesis 5 is particularly controversial.

What conclusions can be drawn? Many individuals who consumed leucine, oleic acid, and DHA lost weight. The loss was not statistically significant in this pilot study. The rate of obesity internationally has increased substantially in recent decades (Bray 2004). The rise in obesity is a major risk factor for heart disease, cancer, diabetes, and other chronic diseases associated with aging (Woo et al. 2001). I have been unable to locate any other double-blind study of a weight loss nutraceutical, despite the wide range of diet products that are commercially available.

This study has resulted in revised hypotheses for nutrient signals likely to contribute to weight loss. For men and young women, 12 g leucine with 6 g olive oil and 6 g fish oil was most effective. For mature women, 12 g leucine and 12 g olive oil were most effective, but individual results were variable, as shown in Table 7. Because DHA and oleic acid appear to have different signal pathways, different combinations and dosages of the two different fatty acids may be effective.

Based on the possible efficacy and demonstrated safety shown in this study, a new study of longer duration with appropriate dosages for young and mature male and female groups is planned.

In conclusion, this study demonstrates that leucine, olive oil, and fish oil may cause weight loss through signaling mechanisms to the brain and adipose tissue. Polyunsaturated fatty acids like oleic acid and omega-3 fatty acids like DHA may have different signaling pathways that can act synergistically.

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