Double-Blind, Placebo-Controlled Trial Examining the Effects of RIAA/Acacia Supplementation on Insulin Homeostasis

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SUMMARY

Inflammatory processes are at the center of many chronic diseases, including metabolic syndrome. One of the hallmarks of metabolic syndrome is the dysregulation in insulin homeo-stasis, i.e., the development of insulin resistance. In the present study, results are reported for the test of a combination supplement in tablet form containing rho-*iso-alpha acids (RIAA) and a proprietary* Acacia nilotica *extract versus placebo in subjects with metabolic syndrome. The primary parameter tested to gauge the effect of supplementation was 2 h post-prandial (2 h pp) insulin level. After 8 weeks of supplementation with the RIAA/*Acacia *combination, a greater reduction in 2 h postprandial insulin levels was observed compared with placebo. Similarly, RIAA/*Acacia *supplementation also led to greater lowering of fasting insulin, fasting glucose, 2 h pp glucose, and fasting triglyceride levels as compared with placebo after 8 weeks. The supplementation also resulted in greater lowering of the homeo-static model assessment (HOMA) score over placebo. All these results taken together suggest that RIAA/*Acacia *supplementation may be of benefit for maintaining normal insulin homeostasis.*

INTRODUCTION

Insulin resistance and/or hyperinsulinemia is characterized by an imbalance in the insulin response, and has been proposed as a preceding factor for myriad conditions, including hypertension, low high density lipoprotein (HDL) cholesterol, hypertriglyceridemia, abdominal obesity, and altered glucose tolerance. Research has implicated dysregulated inflammatory processes in the development of insulin resistance, and several inflammation-induced cytokines have been shown to be expressed during insulin resistance and metabolic syndrome.[1-3] For example, cytokines such as interleukin-1 beta (IL-1β), tumor necrosis factor-alpha (TNF-α), and interferon-gamma (IFN-γ) have been shown to play a role in cytokine induced β cell dysfunction in diabetes.[4-5] Insofar as IL-1β, TNF-α, and IFN-γ are under nuclear factor-kappa B (NF-κB) control, modalities that regulate NF-κB expression may be expected to have a beneficial effect on insulin resistance.

The concept of inflammation and adipocyte interaction in relation to myriad chronic metabolic conditions started with a seminal publication by Hotamisligil et al. in 1993, which demonstrated that adipocytes constitutively express the pro-inflammatory cytokine TNF-α, and that TNF-α expression in the adipocytes of obese animals (*ob/ob* mouse, *db/db* mouse, and *fa/fa* Zucker rat) is markedly increased.[6] Further, neutralization of TNF-α by soluble TNF-α receptor led to a decrease in insulin resistance in these animals. These observations provided a link between an increase in the expression and plasma concentration of a pro-inflammatory cytokine and insulin resistance.

Two botanical substances—hops (genus *Humulus*) and acacia (genus *Acacia*)—have been used in various forms for centuries. For example, published literature indicates that hops, long known to the brewers' art for providing the bitter taste to beers, has anti-oxidant, anti-inflammatory, and anti-carcinogenic activity.[7] *Acacia*, belonging to the family *Leguminosae* and subfamily *Mimosoideae*, are distributed worldwide in tropical and subtropical areas of central and South America, Africa, parts of Asia, as well as Australia. Traditional medicine supports the use of different types of *Acacia* preparations for such diverse indications like sore throat, gingivitis, colitis, diarrhea, bleeding, diabetes, skin diseases, cancer, toothaches, and inflammation in the mouth. Singh et al. reported that a diet of seeds from these *Acacia* plants had hypoglycemic activity in normal rats but not in alloxan-induced diabetic rats.[8] Singh et al., however, did not elucidate whether portions of the plants other than the seed meat (e.g., bark or heartwood, or plant material) extracts have any hypoglycemic activity in normal or diabetic subjects. Aqueous infusions of the seed pods or bark of *Acacia nilotica* have been used in folk medicine for gastrointestinal disorders, while pulverized seeds and pods have been applied to sores of the mouth or to hasten cicatrisation of syphilitic ulcers.[9-10]

We developed and tested a combination product of RIAA from hops (*Humulus lupulus*) and a proprietary *Acacia nilotica* extract on parameters of insulin resistance. In early *in vitro* studies using the 3T3 L1 cell model system, this combination was shown to inhibit the major upstream inflammation-regulating event, the induction of NF-κB, which plays a central role in insulin resistance. In subsequent animal studies using the diabetic mouse model (*db/db*),

this combination reduced insulin levels by 20.2%, which was comparable in efficacy to the positive controls. In this report, we summarize our findings from a human clinical trial using this RIAA/*Acacia* combination on markers of insulin sensitivity in volunteers with the metabolic syndrome.

METHODS AND TRIAL DESIGN

This trial was a randomized, placebo-controlled, double-blind trial conducted at a single study site (the Functional Medicine Research Center). To be enrolled for the trial, subjects (between 18 to 70 years of age) had to satisfy the following criteria: (i) BMI between 25 and 42.5 kg/m^2; (ii) TG/HDL-C ratio ≥ 3.5; (iii) fasting insulin ≥ 10 mcIU/mL. In addition, subjects had to meet 3 of the following 5 criteria: (i) waist circumference > 35 inches (women) and > 40 inches (men); (ii) TG ≥ 150 mg/dL; (iii) HDL < 50 mg/dL (women) and < 40 mg/dL (men); (iv) blood pressure ≥ 130/85 or diagnosed hypertension on medication; and (v) fasting glucose ≥ 100 mg/dL.

Results are reported for subjects who satisfied the inclusion criteria that were randomized to one of 2 arms of the trial: (i) subjects taking the RIAA/*Acacia* combination at 1 serving t.i.d; (ii) placebo, 1-2 servings, t.i.d. The total duration of the trial was 12 weeks. Blood was drawn from subjects at day 1, at 8 weeks, and 12 weeks to assess the effect of supplementation on various parameters of metabolic syndrome.

RESULTS

The initial demographic and biochemical characteristics of subjects enrolled for the trial are shown in Table 1. Both groups began with similar weights, blood pressure, and waist and hip circumferences, indicating that the groups were well matched in these parameters. The initial fasting blood glucose and 2 h post-prandial (2 h pp) glucose values were similar between the RIAA/*Acacia* and placebo groups (99.0 *vs*. 96.5 mg/dL and 128.4 *vs*. 109.2 mg/dL, respectively). In addition, both glucose values were generally within the laboratory reference range (40-110 mg/dL for fasting blood glucose and 70-150 mg/dL for 2 h pp glucose). This was expected, because alteration in 2 h pp insulin response precedes the elevations in glucose and fasting insulin that are seen in later stage metabolic syndrome and frank diabetes.

Fasting blood insulin measurements were similar and generally within the reference range as well, with initial values of 17.5 mcIU/mL for the RIAA/*Acacia* group, and 13.2 mcIU/mL for the placebo group (reference range, 3-30 mcIU/mL). The 2 h pp insulin levels were elevated past the reference range (99.3 *vs*. 80.2 mcIU/mL), and showed greater variability than did the fasting insulin or glucose measurements. Although the initial values were similar, the RIAA/*Acacia* group showed a greater decrease in fasting insulin and 2 h pp insulin, as well as 2 h pp blood glucose after 8 weeks on the protocol (Figures 1 and 2).

Table 1: **Demographic and Baseline Biochemical Characteristics**

	Placebo		RIAA/Acacia	
N	35		35	
Gender				
Male	11 (31%)		12 (34%)	
Female	24 (69%)		23 (66%)	
	Mean	**SD**	**Mean**	**SD**
Age (yrs)	46.0	13.2	47.9	13.4
Weight (lbs)	220.6	35.2	219.5	31.6
BMI (kg/m2)	35.0	4.0	35.4	4.0
Systolic BP (mm)	131.0	15.1	129.7	13.9
Diastolic BP (mm)	83.7	8.5	82.6	7.8
Waist (inches)	42.9	4.9	42.9	4.5
Hip (inches)	47.1	4.0	47.6	3.2
Fasting Insulin (mcIU/mL)	13.2	5.2	17.5	12.1
2 h pp Insulin (mcIU/mL)	80.2	52.1	99.3*	59.2*
Fasting Glucose (mg/dL)	96.5	9.0	99.0	10.3
2 h pp Glucose (mg/dL)	109.2	30.5	128.4	36.9
Fasting TG (mg/dL)	231.2	132.2	255.5	122.5

**One subject was excluded from the analysis due to abnormal 2 h pp insulin values; BMI, Basal Metabolic Index; BP, Blood Pressure; TG, Triglyceride; HDL, High-Density Lipoprotein*

Figure 1: **Effect of RIAA/*Acacia* supplementation on fasting and 2 h pp insulin levels.** For the 2 h pp insulin level assessment, subjects presented after a 10-12 h fast and consumed a solution containing 75 g glucose (Trutol 100, CASCO NERL® Diagnostics); 2 h after the glucose challenge, blood was drawn and assayed for insulin levels (Laboratories Northwest, Tacoma, WA).

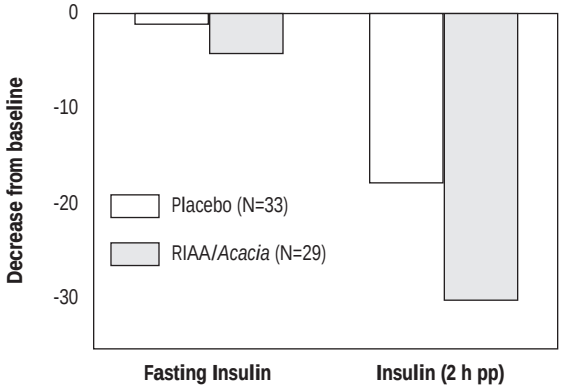
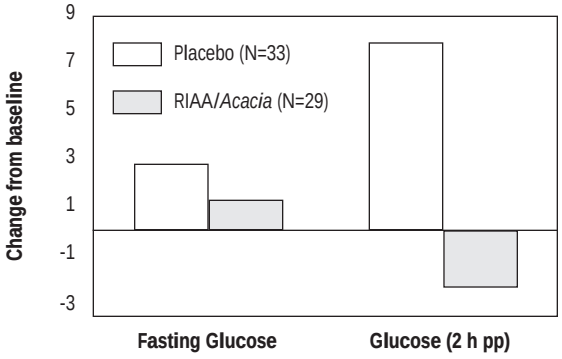


Figure 2: **Effect of RIAA/*Acacia* supplementation on fasting and 2 h pp glucose levels.** For the 2 h pp glucose level assessment, subjects presented after a 10-12 h fast and consumed a solution containing 75 g glucose (Trutol 100, CASCO NERL® Diagnostics); 2 h after the glucose challenge, blood was drawn and assayed for glucose levels (Laboratories Northwest, Tacoma, WA).



The homeostatic model assessment (HOMA) score is a published measure of insulin resistance. The change in HOMA score for all subjects is shown in Figure 3. Due to the variability seen in metabolic syndrome subjects' insulin and glucose values, a subgroup of only those subjects with fasting insulin > 15 mcIU/mL was also assessed. The HOMA score for this subgroup is shown in Table 2, and indicates that a significant decrease was observed for the RIAA/*Acacia* group as compared to the placebo group.

Figure 3: **Effect of RIAA/*Acacia* supplementation on HOMA scores.** HOMA score was calculated from fasting insulin and glucose by published methods [(insulin (mcIU/mL) glucose (mg/dL))/405].

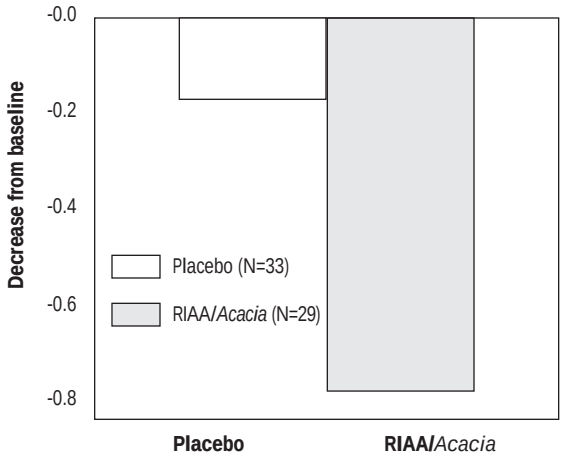


Table 2: **Effect of RIAA/*Acacia* supplementation on HOMA scores in subjects with initial fasting insulin ≥15 mcIU/mL.** The difference between the groups was significant at 8 weeks ($p < 0.05$). HOMA score was calculated from fasting insulin and glucose by published methods [(insulin (mcIU/mL) glucose (mg/dL))/405].

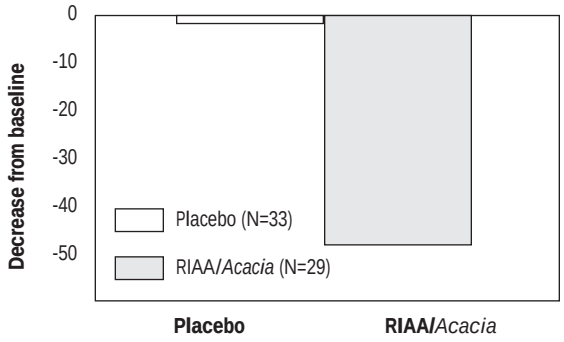
		HOMA Score	
Treatment	**N**	**Initial**	**After 8 Weeks**
Placebo	9	4.39	4.67
RIAA/*Acacia*	13	5.84	4.04

Elevation in triglycerides (TG) is also an important suggestive indicator of metabolic syndrome. Table 3 and Figure 4 indicate that RIAA/*Acacia* supplementation resulted in a significant decrease in TG after 8 weeks as compared with placebo ($p < 0.05$). The TG/HDL-C ratio was also shown to decrease substantially for the RIAA/*Acacia* group (from 6.40 to 5.28), while no decrease was noted in the placebo group (from 5.81 to 5.92).

Table 3: **Effect of RIAA/*Acacia* supplementation on TG levels and TG/HDL-Cholesterol ratio.**

	Fasting TG (mg/dL)			TG/HDL		
Supplementation	**Initial**	**After 8 Weeks**	**Change**	**Initial**	**After 8 Weeks**	**Change**
Placebo	231.2	229.8	-1.4	5.81	5.92	+0.11
RIAA/*Acacia*	258.6	209.6	-49.0	6.40	5.28	-1.12

Figure 4: **Effect of RIAA/*Acacia* supplementation on serum TG levels.**



CONCLUSIONS

Supplementation of metabolic syndrome subjects with a combination of RIAA and a proprietary *Acacia nilotica* extract for a duration of 8 weeks led to greater reduction of 2 h pp insulin levels, as compared to placebo. Further, greater decreases of fasting insulin, fasting and 2 h pp glucose, fasting triglyceride, and HOMA scores were observed in subjects taking RIAA/*Acacia* supplement *vs* subjects taking placebo. These results indicate RIAA/*Acacia* supplementation might be useful in maintaining insulin homeostasis in subjects with metabolic syndrome.

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