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Original article

Effect of novel dietary supplement on metabolism *in vitro* and *in vivo*

Roger A. Vaughan [a, b, c, d, *], Ailish C. White [b], Jason R. Beam [b], Nicholas P. Gannon [c], Randi Garcia-Smith [c], Roy M. Salgado [b], Marco Bisoffi [c, e, f], Kristina A. Trujillo [c, e], Carole A. Conn [d], Christine M. Mermier [b]

[a] Department of Nutritional Sciences, Texas Tech University, Lubbock, TX 79409, USA
[b] Department of Health, Exercise and Sports Science, University of New Mexico, Albuquerque, NM 87131, USA
[c] Department of Biochemistry and Molecular Biology, University of New Mexico Health Sciences Center, Albuquerque, NM 87131, USA
[d] Department of Individual, Family, and Community Education: Nutrition, University of New Mexico, Albuquerque, NM 87131, USA
[e] University of New Mexico Cancer Center, Albuquerque, NM 87131, USA
[f] Biological Sciences, Chapman University, Orange, CA 92866, USA

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ABSTRACT

Obesity is an increasingly prevalent and preventable morbidity with multiple behavioral, surgical and pharmacological interventions currently available. Commercial dietary supplements are often advertised to stimulate metabolism and cause rapid weight and/or fat loss, although few well-controlled studies have demonstrated such effects. We describe a commercially available dietary supplement (purportedly containing caffeine, catechins, and other metabolic stimulators) on resting metabolic rate in humans, and on metabolism, mitochondrial content, and related gene expression *in vitro*. Human males ingested either a placebo or commercially available supplement (RF) in a randomized double-blind placebo-controlled cross-over fashion. Metabolic rate, respiratory exchange ratio, and blood pressure were measured hourly for 3 h post-ingestion. To investigate molecular effects, human rhabdomyosarcoma cells (RD) and mouse myocytes (C2C12) were treated with various doses of RF for various durations. RF enhanced energy expenditure and systolic blood pressure in human males without altering substrate utilization. In myocytes, RF enhanced metabolism, metabolic gene expression, and mitochondrial content suggesting RF may target common energetic pathways which control mitochondrial biogenesis. RF appears to increase metabolism immediately following ingestion, although it is unclear if RF provides benefits beyond those provided by caffeine alone. Additional research is needed to examine safety and efficacy for human weight loss.

1. Introduction

Obesity is an increasingly prevalent morbidity with nearly two-thirds of adult Americans overweight, with over 32% of men and 35% of adult women clinically obese.[1] It is forecasted that roughly 85% of adult Americans will be overweight, over half of which will be clinically obese by 2030.[2,3] Over the past decade, chemical and behavioral interventions that favorably modify metabolic rate have been central to obesity research. Several over-the-counter dietary supplements claim to increase metabolic rate and enhance fatty acid catabolism.

Ripped Freak® (RF) from PharmaFreak (Toronto, Canada) is one such commercially available dietary supplement advertised to act as a thermogenic agent, although there appear to be no previously published data on its efficacy. RF is specifically purported to increase metabolic rate, oxygen consumption, and fatty acid oxidation. RF is also purported to modify signal transduction and induction of genes that control energy homeostasis. Several of the ingredients that purportedly comprise RF's proprietary blend

Abbreviations: GLUT4, Glucose transporter 4; TFAM, Mitochondrial transcription factor A; NRF-1, Nuclear respiratory factor; PGC-1α, Proliferator-activated receptor γ coactivator-1α; REE, Resting energy expenditure; RER, Respiratory exchange ratio; TBP, TATA Binding Protein.

* Corresponding author. Department of Nutritional Sciences, Texas Tech University, PO Box 41240, Lubbock, TX 79409, USA. Tel.: +1 806 834 5285.
E-mail addresses: roger.vaughan@ttu.edu, vaughanr@unm.edu (R.A. Vaughan).

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include caffeine, green tea (綠茶lǜ chá), raspberry ketones, and capsaicin; many of which have been previously linked to increased metabolic rate (Table 1).

Previously, caffeine has been shown to increase metabolic rate in humans and in cell culture.[4–10] Human data suggests that caffeine elicits a dose-dependent increase in resting metabolic rate.[9] In addition, dietary components (such as green tea) have been shown to increase metabolism more effectively than caffeine alone.[4] Dietary supplements similar to RF were previously shown to increase markers of fat mobilization, metabolic rate (measured via indirect calorimetry), and reduce bodyweight and body fat (estimated via Dual-energy X-ray absorptiometry) in healthy young subjects following ingestion.[11–18] Our laboratory recently identified that treatment of cultured skeletal muscle with caffeine can increase both metabolic rate and mitochondrial content in muscle cells, suggesting that commercially available metabolic stimulators may have similar effects.[8] Caffeine is believed to work through phosphodiesterase inhibition leading to increases in cAMP or through increasing cytosolic Ca^{2+}.[8,19,20] In addition, our lab recently showed that treatment with either of two similar over-the-counter supplements, or several other dietary components lead to increased metabolic rate and mitochondrial content in skeletal muscle cells.[8,21–24] We have demonstrated, along with others, that stimulation of metabolism by dietary components induces many molecular adaptations including metabolic gene expression. Specifically, expression of peroxisome proliferator-activated receptor γ coactivator-1α (PGC-1α) is increased following administration of various dietary stimulators of metabolism.[8,22–24] PGC-1α is a transcriptional coactivator that is essential for mitochondrial biosynthesis and acts as a master regulator of energy homeostasis and metabolism.[25–27] Our group has previously reviewed the potential role for dietary components in the stimulation of PGC-1α and corresponding favorable metabolic adaptations for benefit in metabolic disease.[28] Because skeletal muscle contributes largely to total energy expenditure, and because it is highly responsive to a variety of stimuli, skeletal muscle represents a meaningful target for metabolic disease such as obesity and diabetes.[28] This work seeks to evaluate the acute effects of RF on energy expenditure in healthy male human subjects, as well as investigate the molecular effects of RF on skeletal muscle *in vitro*.

Table 1
Purported composition of Ripped Freak®.

Ripped Freak® Formula 766.6 mg[a]
Fat burning metabolism amplifier 400 mg[a]
Methyl EGCG™ (EGCG Derivative Stack) (Green Tea Extract/Camellia Sinensis)
Epigallocatechin-3-O-(3-O-Methyl) gallate Ester (EGCG 3′ ′Me)
Epigallocatechin-3-O-(4-O-Methyl) gallate Ester (EGCG 4′ ′Me)
4′-O-Methyl-Epigallocatechin-3-O-Gallate Ester (EGCG 4′Me)
Epigallocatechin-3-O-(3,4-O-Methyl) gallate Ester (EGCG 3′ ′4′ ′diMe)
4′-O-Methyl-Epigallocatechin-3-O-(4-O-Methyl) gallate Ester (EGCG 4′4′ ′diMe)
Oleuropein Aglycone (Olive Leaf Extract/Olea Europaea)
1,3,7-Trimethyl-1H-Purine-2,6(3H,7H)-Dione Methyl Gallate Ester (Caffeine) (Coffee/Coffea Arabica, Whole Bean)
Uncoupling protein/oxygen utilization amplifier 200 mg[a]
CH-19 Sweet Red Pepper Ester Stack (CH-19 Sweet Red Pepper Extract/Capsicum Annum, Fruit) (0.75% Capsiate)
4-Hydroxy-3-Methoxybenzyl (E)-8-Methyl-6-Nonenoate Ester
4-Hydroxy-3-Methoxybenzyl 8-Methyl-Nonanoate Ester
4-Hydroxy-3-Methoxybenzyl 7-Methyl-Octanoate Ester
Hormone sensitive lipase fat mobilization amplifier 166.6 mg[a]
4-(4-Hydroxyphenyl)-2-Methyl Ethyl Ketone (Raspberry Ketone)
4-(4-Hydroxyphenyl)-2-Butanone Methyl Gallate Ester (Raspberry Ketone – Gallic Acid)

[a] Percent daily value not established.

2. Materials and methods

2.1. In vivo

2.1.1. Human subjects

Healthy male volunteers aged 18–40 years were eligible for the study. Participants were excluded if they were caffeine naïve, clinically obese (BMI > 30 kg/m^2), if they had known cardiovascular disease, hypertension, or refused to adhere to subject-study procedures. Ten ($n = 10$) eligible and willing participants were asked to abstain from caffeine and/or dietary supplement consumption in addition to rigorous exercise at least 48 h prior to each measurement. Participants were also asked not to consume anything but water and not to smoke at least 12 h prior to each study visit. Subject food intake was recorded the day prior to initial metabolic measurements, and subjects were asked to consume approximately the same meal composition 24 h prior to the second measurement. Subjects completed a health history questionnaire, food and beverage recall, and informed consent as approved by the Institutional Review Board (HRPO #13-066) and HIPAA. Participants reported their usual intake of caffeinated beverages (coffee, tea, soft drink, energy drink, etc.) with corresponding serving size, and were asked to list other regularly consumed stimulants (such as those found in dietary supplements or over the counter medications). Subject height, weight, and resting blood pressure were recorded prior to initial metabolic measurement. Body composition was estimated by 3 site skin-fold measurements (chest, abdominal, and thigh) and estimated body density was used to calculate body fat percent using the Siri equation. Descriptive subject data are listed in Table 2.

2.1.2. Human metabolic measurements

Each subject was asked to participate in two trials in a double-blind-placebo-controlled cross-over design consisting of two treatments (one placebo filled with dextrose and one serving of RF constituting the actual treatment) for measurement of resting energy expenditure with at least 48 h between the two measurements. Treatments were provided in a double-blind fashion by a third party. Following anthropometric measurements, the subject was asked to consume a blinded treatment which was followed by a resting blood pressure measurement. Metabolic measurements were taken at baseline, and at both 1 and 2 h following treatment ingestion with blood pressure assessed prior to each metabolic measurement. Resting energy expenditure (REE) and respiratory exchange ratio (RER) were measured using a metabolic measurement system (TrueOne 2400 from Parvo Medics, Sandy, UT) following an overnight fast similar to previous reports.[16] The metabolic cart was calibrated daily per manufacturer guidelines prior to each trial. Participants rested in a supine position in a comfortable ambient temperature in a quiet and dark room. Expired gas was collected by placing a clear hood over the participant's head and upper torso area with plastic seal secured under the subject. Flow rate was monitored by a designated research assistant during the course of the test and maintained at a rate of 1–1.2% expired carbon dioxide per manufacture's protocol. Data were collected for approximately 30 min per measurement and the 5 min duration which produced the lowest variability of REE data was used for values of REE and RER for all tests. REE data were transformed into kcals/kg/day for both total body weight and lean body mass.

2.2. In vitro

2.2.1. Cell culture

Human rhabdomyosarcoma cells (RD) and mouse myocytes (C2C12) were purchased from ATCC (Manassas, VA). Cells were