

**THERMOGENIC EFFECT OF BETA-SYMPATHICOMIMETIC COMPOUNDS
EXTRACTED FROM CITRUS AURANTIUM, IN HUMANS**

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Introduction

The development of obesity is postulated to occur as an interaction between familial and environmental factors. The genetically predetermined abnormalities include a low rate of fat oxidation, which itself may lead to an energy imbalance, and a lower resting energy expenditure (REE) in conjunction with diets high in fat content (1,2,3). Energy expenditure can be thought of as encompassing three components: 1) resting metabolic rate (RMR), defined as the energy requirements for maintaining normal bodily functions at rest, 2) thermic effect of exercise, referring to the increase in energy production of the body in response to physical activity, manifested as heat, and 3) thermic effect of food (TEF), or the increase in energy production post-prandially (4). The TEF is associated with the energy requirements for processing a meal, namely increased activity of ATP-dependent ion channels, active absorption of nutrients, increased protein turnover and increased substrate cycling.

Though the TEF may represent a small portion of the total energy expenditure, some studies have demonstrated defective thermogenesis in experimental rodent models of obesity, while others show reduced thermic response to food in obese human subjects (5,6,7). Hence, TEF could play a role in the development of or maintenance of obesity. More specifically, a blunted thermic response to a meal has been shown to imply a significant energy imbalance in obese individuals (4).

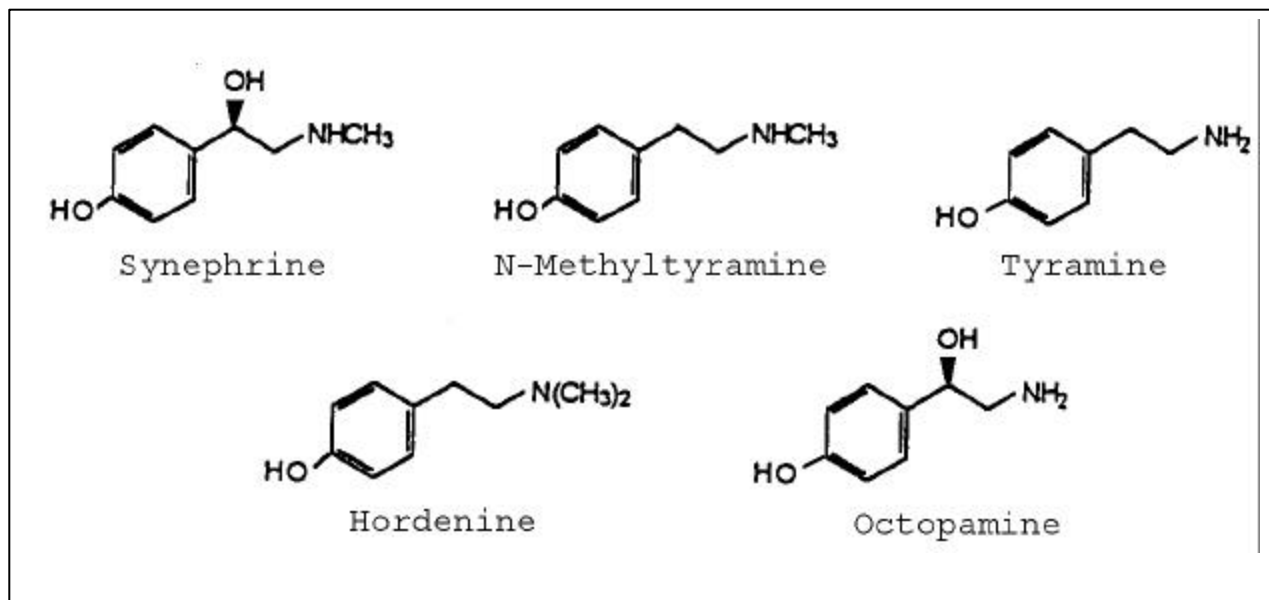
The sympathetic nervous system (SNS), implicated in the regulation of the mobilization of energy reserves, may also be subject to defects thereby predisposing to weight gain (8). Beta-adrenergic receptors are involved in the pathways of lipolysis, glycogenolysis and thermogenesis, with lipolysis and thermogenesis being mediated via the β_3 receptor (9,10). It has

been observed that genetically obese rats exhibit low sympathetic outflow and/or responsiveness in various tissues. In such animal models, sympathicomimetic agents could effectively normalize body weight due to stimulation of thermogenesis in brown adipose tissue via the β 3-adrenoceptor pathway (9).

Studies with the β 3-adrenoceptor agonist BRL 26830A also show a high degree of selectivity for thermogenesis, supporting its potential as an anti-obesity agent (11,12). Hence, the SNS and β 3-adrenoceptor represent another target for intervention in obesity.

Indirect-acting sympathicomimetic compounds potentiate the release of epinephrine and norepinephrine at pre-synaptic sites in the SNS. By acting at β 3 receptors, these catecholamines accelerate the removal of unwanted fat stores by increasing the rate at which fat is released from body stores (lipolysis), while simultaneously increasing the metabolic rate (thermogenesis) (13). Drugs such as Ephedrine, a β -sympathicomimetic, have consistently demonstrated an increase in thermogenesis and improved weight loss in numerous studies (14,15,16,17). Because such drugs simultaneously activate β 1 and β 2 receptors, adverse side effects such as tachycardia, insomnia and tremors are often reported. Interestingly, studies by Dulloo in 1993 suggest that with chronic administration, ephedrine down-regulates adrenoceptor subtypes associated with unwanted cardiac or pressor effects, thereby generating its own selectivity for desirable anti-obesity effects (18).

Zhi-Thin™ is an extract of Citrus Aurantium containing a family of indirect-acting β -sympathicomimetics that stimulate metabolic processes, increase lipolysis and can exert mild hunger-suppressant effects (13). These active adrenergic agents include synephrine, hordenine, octopamine, tyramine and N-methyltyramine, whose structures are illustrated below:



Synephrine has been shown in preliminary studies to be a little more than half as potent as ephedrine, and has reduced central nervous system stimulation. Preliminary non-controlled studies have confirmed that this compound is well tolerated and appeared to enhance weight loss (13). Hence, the thermogenic potential of Citrus Aurantium and other indirect-acting thermogenic substances may be useful in the treatment of obesity, given that obese individuals appear to exhibit reduced SNS activity and a reduced thermic response to food.

The purpose of this study was to assess the acute thermogenic response of Citrus Aurantium at rest in lean and obese individuals. We sought to determine the thermic effect of the alkaloid mixture when consumed orally with water and when consumed with a mixed meal. We also wanted to assess whether the metabolic rate of lean and obese diabetic and non-diabetic individuals would differ in response to the consumption of the alkaloid compound. Our starting hypotheses were as follows:

1. Alkaloids extracted from Citrus Aurantium, particularly Synephrine, induce a notable increase in metabolic rate, when taken orally in a capsule form with water.
2. Citrus Aurantium potentiates the thermic effect of a mixed meal in lean and obese diabetic and non-diabetic subjects during rest.
3. As a result of its sympathicomimetic ability, Citrus Aurantium will cause an increase in serum and urinary concentrations of catecholamines.

Materials and Methods

Seven healthy lean subjects (6 males, 1 female) were recruited to participate in our metabolic study at the McGill Nutrition Centre. All subjects chosen were non-smokers and weren't taking any drugs. Their suitability for participation in the study was assessed by an M.D. in a thorough medical exam, and blood tests for HepB Ag and HIV were performed. Persons with hepatic, cardiovascular, renal or pulmonary dysfunction, gout, or claustrophobia were excluded. Subjects were then informed as to the study protocol, the implications of their participation, as well as potential risks. It was made clear that the process of indirect calorimetry presented no health risk whatsoever, that there was the potential for bruising where the catheter was concerned, and that the total volume of blood drawn represented less than a blood donation. Each of them signed a consent form approved by the RVH Department of Medicine, Human Ethics Committee.

The study comprised three day-long sessions at the McGill Nutrition and Food Science Center. All participants were asked to refrain from eating and drinking, except water, after 8:00 pm the evening before each session. Subjects thus arrived fasted at the McGill Nutrition Center between 7:30 and 8:30 am, and were told to rest in bed for half an hour before the commencement of each experiment. Following this, indirect calorimetry was used to record a 20-minute baseline resting metabolic rate (RMR). The average of the last 15 minutes was used to calculate the 24 hour resting energy expenditure (REE) according to the de Weir equation (19).

Indirect calorimetry was used to determine O_2 consumption and CO_2 production, using the Deltatrac metabolic monitor ventilated hood (Sensormedics, Anaheim, CA). This machine consists of a transparent plastic hood, which is placed over the head while the subject is lying down. A plastic tarp connected to the canopy drapes over the subject's neck and shoulders, and renders the hood airtight when tucked underneath the pillow. A slight negative pressure and a gaseous and temperature steady state are maintained within the canopy throughout the study. Calibrated analyzers in the machine measure minute by minute averages of O_2 and CO_2 concentrations, using air fed by a gas sampling tube directly connected to the upper part of the hood. From this, minute by minute VCO_2 and VO_2 , R.Q. (VCO_2/VO_2) and energy expenditure

are recorded on a printout from the machine, and are expressed as extrapolated values for 24 hours.

The set of conditions following the 20-minute RMR differed between each of the three sessions, and the order of the three sessions was randomized for each subject. On a given day, subjects had their metabolic rates monitored under one of the following three conditions: 1) after consumption of a 392 Calorie mixed meal, over a period of 336 minutes, 2) after consumption of 5 Citrus Aurantium capsules over a period of 300 minutes, or 3) after consumption of both the 392 Cal mixed meal and the 5 Citrus Aurantium capsules, over a period of 336 minutes. During the three sessions, the Deltatrac metabolic monitor was used continuously for periods of 40 minutes, with a 20 minute break per hour.

The mixed meal was presented in the form of two chocolate flavoured food bars (Power 8R, Bariatrix International, Lachine, Quebec), each containing 196 calories of energy. The macronutrient content of the 392 Cal meal, as a percentage of total energy content, is 53% carbohydrate, 29% protein and 18% fat. Metabolic rates were measured for a period of roughly 6 hours so as to obtain 60-70% of the TEF, thereby allowing for comparison of TEF between subjects (4).

Fat-free mass and percentage body fat was measured in each participant by the bioelectrical impedance analysis method, using a 4-terminal bioimpedance analyzer (BIA-103, RJL Systems, Detroit, MI). The procedure and anatomical sites for placement of electrodes was as specified by Lukaski et al. (20). Further anthropometric measures taken included weight (kg), height (cm) and body circumferences (cm). These measurements were taken before the start of the first session only, when subjects were in the fasted state.

Urine was collected during each of the sessions for analysis of urea and catecholamine, (E, NE and DOPA). Prior to measurement of the 20-minute baseline RMR, a "pre-study" urine sample was obtained, against which we compared urinary urea and catecholamines measured in urine collected during the actual TEF measurement. Aliquots of 10ml from each collection were acidified with HCl to a pH of 3, and stored at -70°C until HPLC was used to determine

dopamine, norepinephrine and epinephrine concentrations. An additional 4ml aliquot from each sample was used to obtain the urea concentration by the urease-Berthelot method (21). All catecholamine and urea concentrations were adjusted with respect to the volume of urine produced in each sample, and the time of each sample was noted.

A catheter was inserted into an antecubital vein and kept patent with isotonic saline for blood sampling. Ten cc of venous blood was sampled prior to consumption of the test meal (t=0) and then at 30, 60, 90, 120, 180, 240, 300 and 336 minutes. No blood sampling was done during the session in which the Citrus Aurantium capsules were consumed on their own.

Of the 10cc sampled at each time period, 3cc was placed in a test tube containing trasylol, to be later centrifuged so that serum insulin could be assessed by radio-immunoassay (RIA) using I-125 (No. KTSP-11001). The remaining 7cc of blood was placed into a "green-top" vacuum-sealed test tube containing heparin. After centrifugation, 1cc of plasma was extracted into a test tube for measurement of serum glucose concentrations using the Beckman glucose analyzer and the Beckman glucose reagent kit (No. 671640). The rest of the plasma was put into a test tube containing roughly 10mg sodium meta-bisulphite, for subsequent determination of serum catecholamine concentrations using HPLC. At the time of this writing, all of the serum samples are being stored at -20°C, awaiting testing.

Pulse rates and blood pressures were taken at the beginning of each session and during every break of the TEF measurement. Blood pressure was measured by a Pressurometer auscultatory automated blood pressure system.

Results

In our 7 lean subjects, the 20 minute baseline RMR was found to be reproducible with high reliability between the three days of the test. Also, on any given day of the study, the hourly pulse and blood pressure recordings remained fairly constant with respect to the pre-TEF value taken just after the RMR, indicative of a state of relaxation.

Figure 1 compares the change in respiratory quotient (VCO_2/VO_2) between each of the three conditions, throughout the duration of the study. Each point on the graph represents the average R.Q. for all subjects at a particular point in time. Citrus Aurantium seemed to elicit an increase in R.Q. within the first 50 minutes of the study, both when consumed alone and in conjunction with the mixed meal bars.

Figure 2 compares the thermogenic effect, or increment above resting metabolic rate, of the three conditions. The "meal only" curve represents a typical thermogenic response to a meal, showing a peak metabolic rate within one and a half hours of consumption of the meal, after which the metabolic rate slowly returns to baseline. Of interest here is the increase in magnitude of the TEF peak from 0.88 ± 0.11 KJ/min. to 1.08 ± 0.09 KJ/min. with consumption of the meal and Citrus Aurantium. Also, our results show an increase in energy expenditure with the Citrus Aurantium capsules alone, with a peak of 0.56 ± 0.16 KJ/min ($p=0.024$).

There was a $14.9 \pm 1.0\%$ increase above resting energy expenditure when the capsules were added to the mixed meal, as compared to a $13.5 \pm 1.2\%$ increase above REE with the mixed meal alone. The thermogenic effect of the meal and capsule combination was also greater when expressed as a percent increase above REE and as a percent of the ingested calories (converted to KJ), as illustrated in Figure 4.

Urinary epinephrine increased from 2.25 ± 0.63 nmol/hr to 4.54 ± 0.81 nmol/hour, and dopamine from 50.18 ± 1.68 nmol/hr to 75.39 ± 3.17 nmol/hr ($p,0.05$) after consumption of Citrus Aurantium alkaloid mixture (figure 3). Excretion of norepinephrine showed an insignificant decrease.

Discussion

A minimum of 14 lean, 14 obese subjects with diabetes and 14 obese nondiabetic subjects are required in order to provide an 80% probability of detecting a difference of 8 Calories in metabolic rate with an intra-individual standard deviation of 6.4 Calories. More studies have to be performed in order to bring the sample size to an acceptable number.

Despite this, our preliminary results show trends which support some of our hypothesis with statistical significance. First of all, the significant increase in urinary epinephrine and dopamine excretion are indicative of the sympathicomimetic potential of Citrus Aurantium, for this implies an increased release of these catecholamines into the circulation from presynaptic sites.

Our findings suggest that Citrus Aurantium potentiates the TEF of a mixed meal, as evidenced by an increased thermogenic response in lean subjects after consumption of the mixed meal and Citrus Aurantium combination. In addition, results so far indicate a measurable increase in metabolic rate upon consumption of Citrus Aurantium with water. These early results support the thermogenic potential of Citrus Aurantium. Furthermore, as no irregular changes in pulse pressure or blood pressure were reported, our results indicate that the alkaloid mixture is well-tolerated, and provokes no tachycardia.

It is important to note that obese and obese diabetic subjects have not yet been challenged to the study, however we predict that there might be important differences in responsiveness to Citrus Aurantium in these individuals. The crucial affirmation of the clinical usefulness of this alkaloid mixture will come should the results be the same or greater in these individuals, as we predict.

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