

THERMIC EFFECT OF CITRUS AURANTIUM IN OBESE SUBJECTS

Bhuvan Pathak, Dr. Réjeanne Gougeon; McGill Nutrition Center,
Royal Victoria Hospital, Montreal

Introduction

The chronic problem of obesity, though influenced by many environmental factors, is partly the result of genetic abnormalities leading to a reduced energy expenditure (EE). This lowered EE could result in a positive energy balance and therefore weight gain. EE in all individuals can be divided into three categories: (A) resting metabolic rate (RMR), (B) post-prandial thermogenesis or the thermic effect of food (TEF), and (C) the thermic effect of exercise. One factor which contributes to a lower EE is a low RMR, and another is a low TEF. TEF is defined as the acute increase in energy expenditure above the RMR after energy intake (1,2). Although TEF represents a relatively small fraction of total energy expenditure in lean individuals (10% of EE) (3), this value has been reported in some studies to be significantly lower in obese individuals (1,4,5). It has also been shown that thermogenesis remains at lowered levels even in post-obese subjects compared with weight-matched controls, thus proving that lowered TEF might be a significant factor in the pathogenesis of obesity (4,6).

One abnormality which causes this lower TEF is found in the T3 hormone derived from the thyroid which increases the proton permeability of the inner mitochondrial membrane and therefore leads to a decrease in the efficiency of energy production (7).

A second and very important defect may be found in the sympathetic nervous system (SNS); more specifically decreased SNS activity and/or responsiveness of various tissues to SNS activity. The decreased responsiveness to SNS activity is concentrated at the β -adrenoreceptors which mediate (among other things) lipolysis, thermogenesis, and glycogenolysis, and especially at the β -3 receptors which mediate lipolysis and thermogenesis (8,9).

Since obese individuals seem to exhibit reduced SNS activity and thus a reduced TEF, an increase in energy expenditure (EE) through the mediation of thermogenesis may create a negative energy balance. This negative balance would favor weight loss and would be of clinical significance as an adjunct in the treatment of obesity, or could help prevent further weight gain.

Although β -adrenergic drugs may successfully increase the rate of thermogenesis, many undesirable effects may also be obtained due to the coupled stimulation of other β -adrenoreceptors. For example; ephedrine, a sympathomimetic alkaloid, enhances the thermogenic effect in an individual over time but at the same time presents negative effects on heart rate and blood pressure and thus cannot be used in patients suffering from hypertension (10). Other substances, however, have proved to increase the TEF while maintaining relaxed heart-rate levels. One such substance is a mixture of ephedra (containing both ephedrine and pseudoephedrine and known as MaHuang in Chinese medical literature) and caffeine which achieved the above-mentioned results in lean, obese, and obese-diabetic persons (10).

Citrus Aurantium (C.A.) has also already been tested in *lean* individuals and has shown encouraging results; the TEF of a mixed meal (in the form of two food bars) was enhanced when taken with C.A. in a pill form (11). The pills taken alone also caused a small but significant increase in EE. C.A. caused increased respiratory quotient (VCO_2/VO_2) values within the first fifty minutes of the study when consumed alone as well as conjointly with a mixed meal. Finally, urinary catecholamines including dopamine and epinephrine increased when the C.A. was consumed, while norepinephrine excretion did not change significantly.

The purpose of this study is to evaluate whether alkaloids extracted from the immature fruit of C.A. induce a significant increase in the metabolic rate when taken orally in the capsule form with water, and potentiate the thermic effect of a mixed meal in (otherwise healthy) *obese* subjects during rest. The extract of C.A. (labeled as Zhi-Thin) contains small amounts of synephrine and octopamine which are direct and indirect-

acting adrenergic agents with β -agonist activity thus potentially increasing lipolysis and fat oxidation (12).

The original hypotheses for this study remain consistent with those of the C.A. study done in lean subjects:

1. Alkaloids extracted from Citrus Aurantium, particularly synephrine, induce a notable increase in the 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 sympathomimetic ability, Citrus Aurantium will cause an increase in serum and urinary concentrations of catecholamines.(11)

Materials and Methods

Subjects:

The majority of the subjects recruited for this study were selected from a pool of subjects previously involved in an eight-week study concerning the effect of a reduced caloric diet in conjunction with C.A. or placebo on weight loss. Their clinical characteristics are shown in Table 1. Five women were recruited; they underwent a thorough medical examination to assess whether they were appropriate candidates for the study. This meant that the obese subjects had to be devoid of health problems such as diabetes, high blood pressure, liver and renal disease, etc.... The subjects also had their heart rate and blood pressure taken, and were screened for HIV and Hepatitis B viruses to ensure the safety of those involved in the study. Finally, the subjects were made aware of the minimal risks associated with participation in the study such as bruising with the insertion of the catheter (required for the withdrawal of a total volume of blood that equaled less than that of a blood donation) and possible allergic reactions to the food bar used as a controlled mixed meal. The subjects were also told not to expect any weight loss since the study was measuring the acute effect of C.A. over a few hours rather than the prolonged effect over a few weeks or months. The women were then required to sign a consent form approved by the Royal Victoria Hospital's Department of Medicine

Human Ethics Committee prior to the start of the study. The subjects were non-smokers at the time of the study, and had other similar habits (occasional alcohol and moderate coffee drinking for example).

Characteristic	Value
Age (yrs)	49.4 ± 4.6
BMI (kg/m)	34 ± 5.8
Waist-Hip Ratio	0.81 ± 0.07
Arm Circumference (cm)	35.1 ± 4.1
Weight (kg)	79.8 ± 14.5
Fasting np R.Q.	0.81 ± 0.02
%Body Fat	43.2 ± 9.2
RMR (kilocalories)	1247 ± 114

Anthropometric Measurements:

The subjects had their height and weight measured, and their percent body fat was determined using bioelectrical impedance analysis (BIA) (13,14) followed by calculations using the Lukaski equation (15). BIA is a painless, non-invasive procedure which sends electrical impulses through the body via electrodes. BIA measures the different values of resistance to electrical current caused by different body concentrations of materials such as fat and water. Resistance from BIA readings were taken using two different instruments, namely the BIA-103 (RJL systems, Detroit, MI) and the Tanita Bodyfat Analyzer (model TBF-105) to ensure accuracy and consistency in the readings. Finally, body circumferences including hip, waist, and upper arm were taken.

The Thermic Effect:

On all three mornings of the study the subjects arrived at the Nutrition Center in the fasted state, and were asked to rest in a chair or bed for approximately half an hour to allow their metabolic rate to return to its resting level. The resting metabolic rate (RMR) was then measured for twenty minutes with an indirect calorimeter, Deltatrac Metabolic Monitor (Sensor Medics, Anaheim, CA). Indirect calorimetry involves the placement of a transparent, plastic canopy over the head and shoulders of the individual. The subject,

in the supine position, must not make any sudden movements and must breathe normally while the calorimeter measures the minute by minute O₂ and CO₂ changes used to calculate the respiratory quotient (R.Q.) and RMR/24hrs (16). An average of the last fifteen minutes is taken as a value for the subject's RMR. Heart rate and blood pressure were then taken, after which the subjects were asked to eliminate and discard any urine prior to the intake of foods and/or C.A.

There were three different protocols which then followed (one for each of the three visits) and they were performed in random order for each subject. The studies were named as follows:

- Thermic Effect of Food #1 (TEF 1): the effect of a mixed meal only
- TEF 2: the effect of C.A. only
- TEF 3: the effect of the mixed meal in conjunction with C.A.

The mixed meal was administered in the form of two PWER8EXP BAR POWER THERM food bars. PWER8EXP contained a total of 209 (874 kJ)calories with 29% derived from proteins (15.3g), 48% from carbohydrates (27.3g), and 22% from fats (5.0g) (17).

Treatment:

The subjects were given the appropriate products to consume (according to the study being done) and were placed under the ventilated hood for 40 minutes. At the end of the 40 minutes, they were given a 20 minute break during which they were asked to refrain from moving too much, and during which their heart rate and blood pressure were measured. This cycle was repeated until the effect of the consumed products wore off causing the RMR to return more or less to its baseline value (usually six cycles for TEF 1 and 3, and four cycles for TEF 2).

Analytical Methods:

Furthermore, all pre-study (overnight) urines as well as study urines were collected and preserved using 6M HCl added to a pH of 2-3. Aliquots were then stored at -20°C and -70°C to be tested for urea and catecholamines (epinephrine, norepinephrine, and dopamine) later on.

Also, for TEF 1 and 3, 10 ml samples of blood were taken from the subject at $t=0'$, 30', 60', 90', 120', 180', 240', 300', and 360'. Seven of the 10 ml were placed in green-capped heparin vacuum tubes to test for catecholamines and plasma glucose, while the other three ml were transferred to tubes containing trasylol to measure insulin. The aliquots to be tested for catecholamines were stored at -70°C (while those to be tested for glucose and insulin were stored at -20°C). The Beckman Glucose Analyzer (No. 671640) (18) was used to read plasma glucose levels.

The subjects were given a regular hospital meal at the end of each of the three studies.

Statistical Analysis:

The data were analyzed by calculating the surface area under the curve of the RMR graph and subtracting it from the area under the TEF curve. This value represented the effect of the given treatment and was used to calculate the percent increment above RMR.

Paired t-tests were then done to determine the significance of the values obtained for the percent increments and percent of the meal consumed. Finally, unpaired t-tests were done to validate comparisons between pre and post study catecholamines.

Results

Figure #1 describes the TEF or the increase in energy expenditure above the baseline RMR. Each line represents one of the three different studies, as well as the means and standard error of the mean of five subjects. Each point represents the average measured EE every eight minutes of the protocol. The effect of food intake alone (TEF 1) on RMR reaches a peak at 30 minutes and then begins to decline gradually to baseline value, but never reaches it within the allotted time of the study (6 hours). This was also shown in studies done by Reed and Hill (1). On the other hand the increment effect of the meal given with C.A. (TEF 3) also reached a peak at 30 minutes but then energy expenditure continued to increase to an even greater value fully reached at roughly 72 minutes to taper off, not reaching baseline within the 6 hours of study. Finally, the intake of C.A. alone (TEF 2) was associated with an increase in EE above RMR that peaked at 30 minutes and gradually returned to baseline within 4 hours.

The thermic effect of the meals expressed as percent increment above RMR is shown in Figure #2. We found that the thermic response of the meal was significantly greater with the concurrent intake of Citrus Aurantium (18.3% meal plus C.A. compared with 13.8% meal alone, $p=0.03$) which represented a significantly greater contribution of the energy content of the meal (12.7% with C.A. compared with 9.6%, $p=0.027$).

Figure #3 compares the increase in (R.Q.) in all three studies. Data are plotted as mean and standard error of the mean of every eight minutes. The response reflects that of the TEF with meal only (TEF 1); the R.Q. increased from 0.80 to 0.89 in roughly 30 minutes and then returned to the baseline value within 6 hours. With meal and C.A. (TEF 3) R.Q. increased significantly to a higher value of 0.91 within 88 minutes and then returned to baseline within 6 hours. C.A. (TEF 2) was associated with a slight increase from 0.81 to 0.83 within 30 minutes and then a return to the resting levels.

Finally, Figure #4 shows urinary excretions of epinephrine, norepinephrine, and dopamine in nmol/hr for and before the study with C.A. alone (TEF 2). Only epinephrine excretion increased significantly ($p=0.012$) from 0.352 nmol/hr without C.A. to 0.787 nmol/hr with the alkaloid. Excretions of norepinephrine and dopamine did not change significantly.

It is also important to note that the heart rates and blood pressures of all the subjects remained constant throughout the entire study at levels equal to those taken at baseline.

Discussion

The results of this study indicate that C.A. exhibits sympathomimetic behaviour in obese subjects. Indeed, intake of the alkaloid substance was associated with greater thermic response to a mixed meal. Furthermore, C.A. when taken alone caused a measurable increase in RMR within 30 minutes thus indicating that the compound causes an increase in energy expenditure above baseline. The slight increase in R.Q. was atypical of the proposed situation since R.Q. values would decrease with increased lipolysis.

Secondly, the increased urinary excretions of epinephrine were indicative of the stimulatory effect of C.A. The compound acted in a sympathomimetic fashion thus increasing the mobilization of epinephrine.

Finally, since the heart rates and blood pressures of all the subjects remained stable, C.A., at such doses, does not appear to be associated with unfavorable effects especially for individuals with hypertension. Therefore, C.A. may selectively activate certain β -adrenergic receptors in charge of lipolysis and thermogenesis.

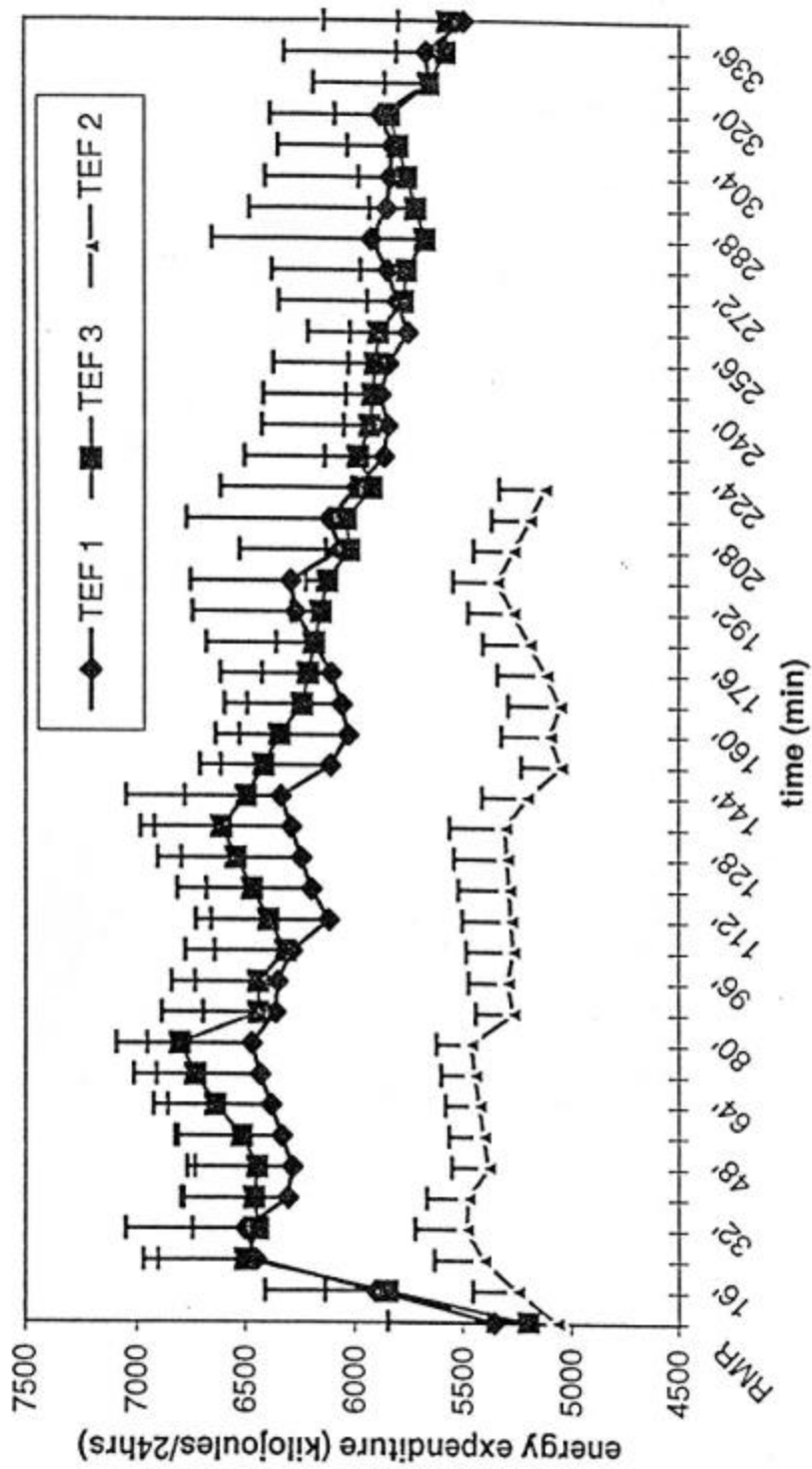
The studies done with C.A. to date have involved healthy lean and obese subjects. In both populations, the thermic effect of a mixed meal expressed as a percent increment above RMR was significantly increased when taken with C.A. ($p=0.040$), despite the small number of subjects studied. More obese non-diabetic subjects need to be studied if they are to be compared to the lean population, as well as to affirm whether the R.Q values actually increase or decrease (the studies are ongoing at the present time). Furthermore, the effect of C.A. will be studied in obese type 2 diabetic persons in order to further evaluate the effectiveness of this compound in that population.

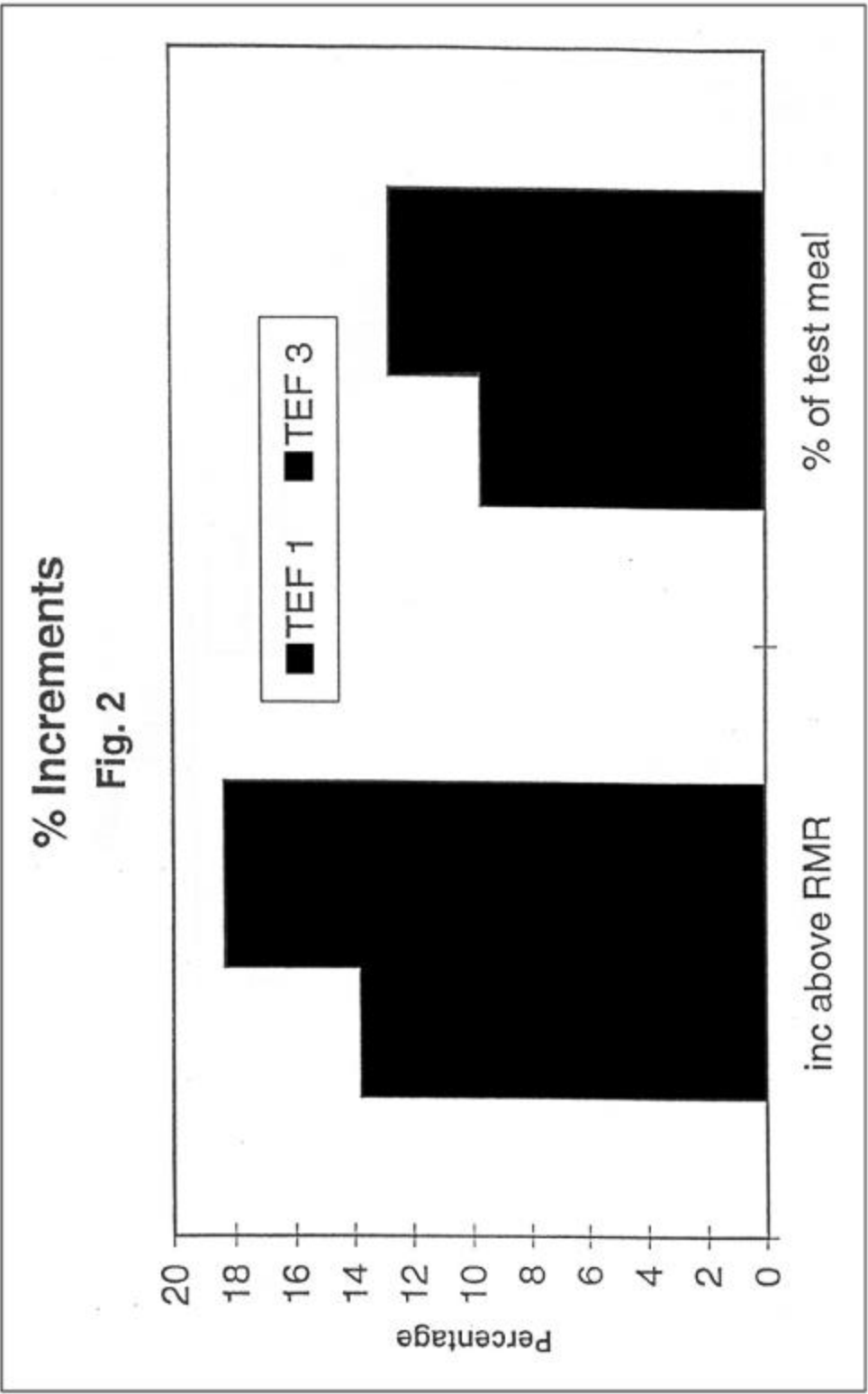
It is conceivable that the effect may be detectable or amplified in individuals characterized by central abdominal obesity. Although the increment in heat production was significantly greater in our subjects when given citrus aurantium, the increase in energy expenditure represented an average of 12 kcalories i.e.. 12.7 compared with 9.6 % of 418 kcalories, the energy content of the meal, or 53.1 compared with 40.1 kcalories. The effect was obtained early after intake of C.A at the maximum dose recommended, as shown in lean subjects in whom the response appears to be of a lower magnitude.

This study shows that C.A. may increase the thermic response of a high protein meal by 4%, a value that could represent 60 kcalories per day. The latter as well as the clinical benefit for successful weight control of such increment remain to be demonstrated.

Thermic Effect of Food

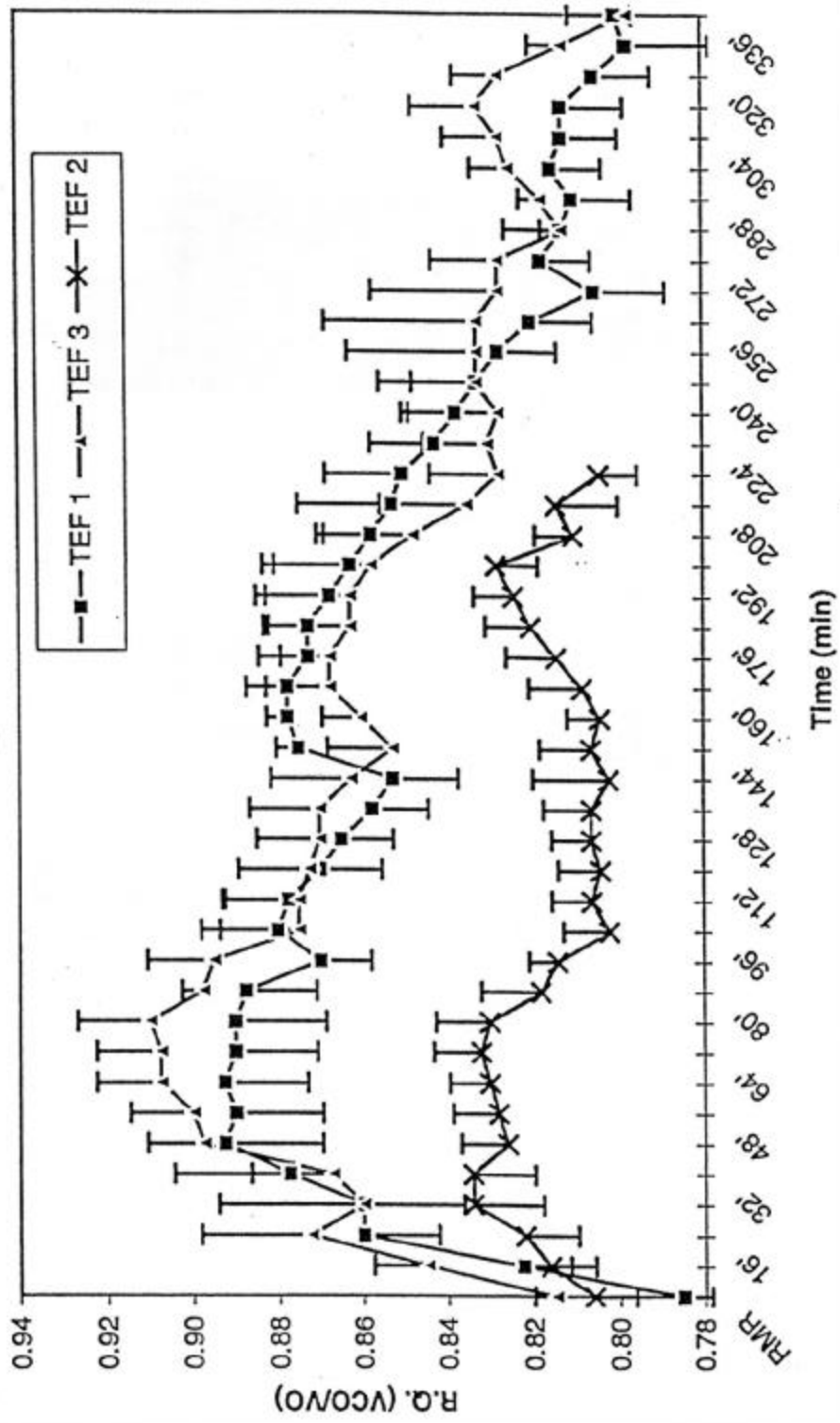
Fig.1

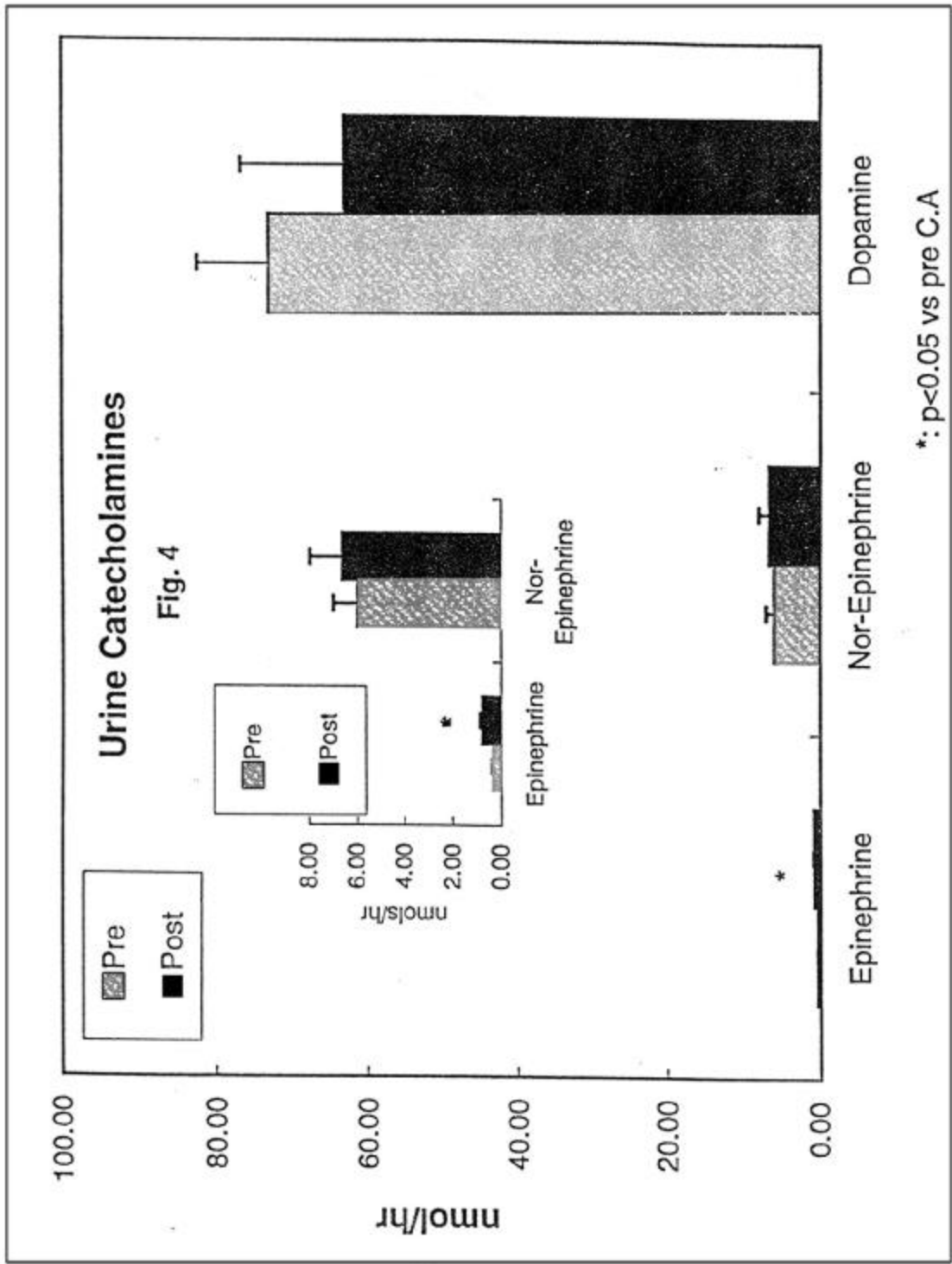




Respiratory Quotient

Fig. 3





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