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Validated Fat-Oxidation Rates in Postmenopausal Women

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Abstract

Background: During menopause and throughout aging, women are at an increased risk for a plethora of chronic conditions many of which are related to the interruption of estrogen. The primary and secondary prevention benefits of habitual unstructured and structured physical activity (PA) are well documented. Fat oxidation rates (FOR) at rest and during exercise have been reported to decrease though the menopausal transition.

Methods: Twenty-three middle-aged Middle Eastern (n=11) and Caucasian (n=12) women were recruited. An independent t-test was used to determine differences between the groups for: anthropometrics, menstrual characteristics, resting blood pressure (BP) and heart rate (HR), PA levels, blood lipid panel, blood hormone panel, glucose, resting VO_2, and VO_{2peak}. A subset of participants had their VO_2 and maximum fat oxidation rate (FOR_{max}) determined on three separate occasions to assess the reliability of the protocol.

Results: No significant differences were observed between FOR at rest or during exercise, and/or other physical and physiological parameters. There were no significant differences between the three separate trials for the VO_2 and FOR_{max} values ($P = 0.565$).

Conclusions: This study contributed to the ever-growing body of literature on FOR and women's health by exploring an ethnic group that has not been previously studied.

Keywords: Cardio-Pulmonary Physiology; Fat Metabolism; Fat Oxidation; Weight Loss; Obesity Prevention; Menopause

Background

During menopause and aging, with changing hormone levels, women are at an increased risk of chronic conditions such as, cancer, type-2 diabetes, autoimmunity, osteoporosis and cardiovascular diseases [1,2]. The risks related to post-menopause are mainly due to the cessation of estrogen, which has indirect protective effects on lipid, glucose metabolism and direct effects on blood vessel function. Thus, post-menopause is commonly associated with hypertension, an increase in overall body mass and altered adiposity distribution, and the most frequently related risk-factors of coronary artery disease [3]. Hypertension is due to increased body mass index (BMI), with insulin-resistance, sodium retention, increased blood viscosity and estrogen deficiency with increased smooth muscle cell proliferation, which causes an increase in systemic vascular resistance. Age and estrogen deficiency together are the most important causes of cardiovascular risk in post-menopause [4]. Contrastingly, the benefits of unstructured physical activity (PA) have been well established, and promoting increased levels of PA to prevent and or treat a number of chronic diseases (obesity, diabetes, cardiovascular disease) dates back at least to the 1950s [5-10]. Estrogen, progesterone and testosterone levels have been reported to influence substrate metabolism and more specifically fat metabolism at both rest and during exercise [11,12]. As these hormone levels change during the menopausal transition they may be contributing to the increase in overall body mass and visceral adiposity distribution observed post-menopause.

Fat Oxidation Rates (FOR) at rest and during exercise have been reported to decrease throughout the menopausal transition and therefore the decrease in estrogen has been associated with decreases in FOR at rest and during PA [11, 12]. In addition, differences in hormones levels [13,14] and FOR [15] have been observed among White, African American, European and South Asian ethnic groups. Further, differences in FOR have been previously observed between select ethnic groups and the respective post-menopausal hormonal modifications have been speculated to influence these differences [16].

FOR is the difference between the rate of oxygen oxidation and synthesis from carbohydrates. Frayn's equation considers both of these variables and thus is often used for the determination FOR_{max} [17]. FOR and the intensity that elicits FOR_{max} were commonly determined by a validated graded exercise (GXT) test (GXT) on a cycling ergometer via indirect calorimetry) [18]. Treadmill based GXT protocols have become more popular as they may lead to a more accurate measurement of FOR_{max} [19]. Achten and colleagues tested three (GXT) protocols against continuous exercise and concluded that a protocol consisting of 35 W increments and 3-minute exercise steps provides a valid assessment of FOR_{max} in well-trained individuals [18].

In health-oriented PA interventions, it is often reported that reaching FOR_{max} may be associated with most efficient weight reductions, although limited experimental evidence exists. Further insight into FOR_{max} and its reliability would allow for it to potentially be used as a target within customized PA interventions. The purpose of this study was to i) validate the treadmill based indirect calorimetry FOR procedures and ii) compare resting FOR, FOR during incremental exercise and $FOR_{max,}$ hormone levels of Middle Eastern (ME) and Caucasian (C) post-menopausal women for further understanding of the menopausal transition and the effects on FOR.

Methods

Study Participants

The experimental protocol conformed to the standards set by the Declaration of Helsinki and was approved by York University's Research Ethics Board. All the women provided written Informed Consent to their voluntary participation in the study. Twenty-three participants were recruited, including eleven ME post-menopausal women via snowball technique. The inclusion criteria indicated that participants must be post-menopausal and otherwise healthy, not on metabolic altering medications or hormone replacement therapy, and had not undergone surgically induced-menopause. Menopausal status was based on the participant's self-report, having no menstrual cycles in the past 12 months, confirmed by their FSH level, >30 mIU/mL with the blood draw. Participants were screened by a qualified exercise physiologist using the evidence based tools, the PAR-Q+ and ePARmed-X+ (www.eparmedx. com) for contraindications and risk stratification. Pre-exercise Heart Rate (HR) and blood pressure were measured using the BpTRU100 (Surgo Surgical Supplies, Toronto, Ontario) automated device to ensure participants were within an acceptable range prior to the initiation of the exercise protocols; blood pressure < 160/90 mmHg. Six measurements were taken consecutively with a 1-minute rest interval in between, and averaged. Participants were grouped based on their ethnic origin ME or C, which was determined by their birthplace.

Experimental Protocol

Participants reported to the York University Human Performance Laboratory on three separate occasions. Pre-exercise screening took place on the first experimental day. The first visit consisted of pre-screening plus an incremental-to-maximal effort treadmill test for the determination of aerobic fitness or power (VO_2max) using the criterion open circuit spirometry discrete system. On the second experimental day, the study participants underwent the fat oxidation protocol following an 8-10 hour overnight fast and 500 mL of water. The FOR protocol was a modified version of Achten, Venables and Jeukendrup treadmill protocol (19). The workloads were the same duration in length and the increase in intensity at each new stage was consistent with the protocol, but the current protocol was walking in nature, rather than running. The third day was used to perform a second trial of the FOR protocol, the same starting speed and loading sequence were used, duplication of the first trial was attempted. The FORmax values of the two trials were used in the analysis and assisted with determining the reliability amongst the criterion open circuit spirometry system. A further description of the visits and the types of exercise performed are detailed below.

Visit 1

Anthropometric data including height, body mass, body fat percentage, skinfolds, and waist circumference were collected using standardized laboratory protocols (20). Body mass was measured upon each visit using the Seca Alpha Scale (Modell 770, Germany). Body fat percentage was measured, without shoes, using bioelectrical impedance analysis (Tanita scale, model TBF-612, Arlington Heights, IL). Height was measured without footwear, using a wall-mounted stadiometer. Waist circumference was measured following the standard National Institutes of Health protocol, with a tape measure around the waist, on the skin, at the level of the iliac crest. Skinfolds (triceps, biceps, subscapularis, iliac crest, and medial calf) were measured using Harpenden fat calipers (Baty International, Burgess Hill, UK) according to the PALM protocol to determine body adiposity [20].

The incremental-to-maximal effort treadmill test for the determination of VO_2max followed the same loading sequence for all participants. The protocol included a 2-minute warm-up, and then progressive exercise workloads increased every two minutes (treadmill speed and/or elevation). The participants were instructed to remain on the treadmill until their work tolerance was compromised, at which point they received a 2-minute low intensity active recovery period. Following recovery, the participants completed another incremental workload that was again followed by a 2-minute recovery. This discontinuous sequence was repeated until the VO_2 of the subsequent workload was, equal to, or lower than the previous, indicating attainment of VO_2max (20-23). The VO_2 was determined from measurements obtained during the last 30 seconds of each workload via direct analysis of mixed expired gases.

The discrete component system consisted of; a 120L Tissot gasometer (Warren E Collins LTD, Braintree, MA), rapid response oxygen and carbon dioxide gas analyzers (Applied Electrochemistry, Model S-3A and CD-3S, Sunnyvale, CA), a flexible plastic hose, two-way y-valve (Ewald Koegal Co, San Antonio, TX), mouthpiece, and nose plugs [22]. Once the expired gases were collected in the Tissot, they were then analyzed using the gas analyzers. The collected variables; minute ventilation, fractions of expired carbon dioxide and oxygen, were then used to calculate the participants' VO_2max. The criterion HR was measured throughout using a Polar HR chest monitor (Polar Electro, Kempele, Finland).

A separate cohort of participants (n = 9, 5 males and 4 females) had their VO_2 and FOR_{max} determined using the open circuit spirometry discrete system on three separate occasions to assess the reliability of the protocol and the measurement of FOR_{max}.

Visit 2

The study participants arrived to the laboratory following an 8-10 hour overnight fast. Blood samples were drawn from each participant at the beginning of the second visit while in a fasted state to characterize the blood lipid, sex hormone and glucose levels. The blood extractions were performed by a certified phlebotomist. Once the blood was obtained from the participants, the vials rested for 30 minutes before being spun in a centrifuge for 15 min. After the samples were spun the plasma was transferred to storage vials to be sent out to an external laboratory (Canadian Life Labs). The blood lipid panel measured; HDL-cholesterol, LDL-cholesterol, triglycerides, total cholesterol, and the HDL-cholesterol to total cholesterol ratio was calculated to ensure normal blood lipid levels for each participant. The blood sex hormone panel analyzed; estradiol, estrone, progesterone, testosterone, SHBG and androgen index, which was calculated using the values from the testosterone and SHBG, FSH, LH,

and insulin. Urine samples were obtained to measure cortisol levels. The urine samples were taken on the same morning as the blood draws, while in a fasted state. The sample was collected in sterile urine cups and transferred to vials to be sent to an external laboratory (Canadian Life Labs) along with the blood samples for analysis.

Following the blood and urine sample collections, the study participants underwent the modified Achten, Venables and Jeukendrup walking fat oxidation protocol. Resting and exercise HR was measured via Polar chest strap HR monitor (Polar Electro KP 4, Kemple, Finland). Resting VO_2 and FOR measurements were obtained with the participant seated in a chair. Expired gas was collected and analyzed; in the same manner as during the GXT test and then used in the formula to calculate FOR. The protocol began at 1.5-1.8 mph and 1% incline, depending on the comfort and height of the participant. Speed increased by 0.2 mph every 3 minutes until the participant was at a brisk walking pace (3.4-3.6 mph). Once the maximum walking speed was attained, the incline increased by 2% every 3 minutes thereafter, until a respiratory exchange ratio of 1.0 or greater was obtained. In the last 30-60 seconds of the workload the expired gases were collected for the calculation of FOR. At the end of each workload both participants' HR and the rating of perceived exertion score were obtained and were used to gage the participants' work intensity. Frayn's equation for the calculation of FOR was employed to measure substrate oxidation at each workload using indirect calorimetry. Urinary nitrogen excretion rate was assumed to be negligible for the purpose of the calculations.

The equation employed is as follows:

$$\text{Fat } (g \cdot min^{-1}) = 1.67 * VO_2 \ (L \cdot min^{-1}) - 1.67 * VCO_2 \ (L \cdot min^{-1})$$

FOR was expressed relative to fat-free mass and body mass for each participant at each workload. The FOR relative to fat-free mass were used to create the FOR curves for each participant and the analysis of resting and FOR_{max}.

Visit 3

The third day was used to perform a second trial of the FOR protocol, the same starting speed and loading sequence were used, duplication of the first trial was attempted. The FOR_{max} values of the two trials were used in the analysis and assisted with determining the reliability amongst the criterion open circuit spirometry discrete system.

Statistical Analysis

The study participant characteristics were expressed as means and standard deviations (X±SD). A total of n=24 study participants were recruited, 12 per group. One middle-eastern women was not able to continue with experimental protocol, thus a total of n=12 C and n=11 ME were used for the statistical analysis. An independent t-test was used to determine differences between the ME and the C participants anthropometrics, menstrual characteristics, resting BP, resting HR, PA levels, blood lipid panel, blood hormone panel, glucose, resting VO_2, and VO_{2peak}. A paired t-test was performed on the two trials of the FOR trials to determine any differences between the two occasions. Regression analysis was performed on the all the hormones and other measured variables against WC and FOR for each group. Statistical analysis was conducted using a standard statistical software program, SPSS 27. The reliability of the protocol was determined by testing for its coefficient of variation using the repeated measures ANOVA –and intra class correlation coefficient (ICC) when it was reproduced on the same individual on three separate days. The ANOVA was calculated using the Excel two- factor without replication formula. The ICC was calculated using the IBM SPSS Statistics Data Editor Software. The two-way mixed model, and the consistency type formula were used.

Results

There were no significant differences between the three separate trials for the VO_2 and FOR_{max} values (P = 0.565). The correlation between the two trials was r=0.97 ± 0.03. The ANOVA calculations revealed that there was a significant difference between individuals in terms of FOR_{max} (p=0.000073, F=9.7), but this was expected given variability between individuals. Within the same individual, when the protocol is conducted on three different days there is no significant difference (p=0.60, F=0.52). The ICC analysis on FOR_{max} (r=0.893) and $\%VO_2max$ or VO_2peak (r = 0.793) indicate good reliability. This permitted the application of the treadmill based GXT protocol coupled with indirect calorimetry to be used as the criterion method during the remaining exercise sessions and comparisons.

Table 1 contains the anthropometric, physical plus physiological fitness profiles and menstrual plus age measures for all of the female study participants (12 C, 11 ME). Significant differences between the C and ME groups where observed for the following study participant characteristics; mean age of

the ME was younger (p= 0.004), ME group observed their first period at an earlier age (p= 0.023) and had their last menstrual cycle early in life (p= 0.031), menstrual age (age of last cycle - age of first cycle) was shorter in the ME group (p= 0.007), ME had a higher body mass (p= 0.027), a higher BMI (p= 0.32) and a larger NIH waist circumference measurement (p= 0.005).

Table 1: Anthropometric, Physical plus Physiological Fitness Profiles and Menstrual Measures of the Study Participants

Characteristics	**Cauciasian (C)** (n=12; X±SD)	**Middle Eastern (ME)** (n=11; X±SD)	**P-value**
Age (yrs)	57.1± 3.3	52.6 ± 2.8	0.04
Age of First Cycle (yrs)	12.2 ± 8.2	13.3 ± 1.1	0.023
Age of Last Cycle (yrs)	51.1 ± 2.6	46.5 ± 5.7	0.031
Menstrual Age (yrs)	39.0 ± 2.2	32.2 ± 5.6	0.007
Height (cm)	161.5 ± 4.6	162.2 ± 5.9	-
Body mass (kg)	71.0 ± 12.1	84.0 ± 16.7	0.027
BMI	27.0 ± 3.8	31.8 ± 5.9	0.032
NIH Waist Circumference (cm)	90. 9 ± 11.0	106.1 ± 11.2	0.05
Sum of 5 Skinfolds (mm)	107.5 ± 35.6	136.1 ± 49.3	-
Tanita Body Fat Scale (%)	37.0 ± 5.4	41.7 ± 6.4	-
Resting Blood Pressure (mmHg)	109/72 ± 12/11	114/72 ± 13/9	-
Resting Heart Rate (bpm)	67 ± 7.2	72 ± 12	-
Resting VO_2 Absolute ($LO_2 \cdot min^{-1}$)	0.26 ± 0.09	0.33 ± 0.05	-
VO_2 peak Absolute ($LO_2 \cdot min^{-1}$)	1.98 ± 0.3	1.78 ± 0.3	-
Peak Heart Rate (bpm)	164 ± 9	158 ± 10	-

- p>0.05 no significant difference

Table 2 contains the sex hormone and blood lipid results of the C and ME participants with t-test for differences between groups. No significant differences were not detected between the two groups, indicating that there are no hormonal variations between these two ethnicities.

Table 2: Blood Test Results of the Study Participants

Characteristics	**Caucasian (C)** (n=12; X±SD)	**Middle Eastern (ME)** (n=11; X±SD)
Estradiol (pmol/L)	84.93 ± 31.38	71.27 ± 4.55
Estrone (pmol/L)	138.25 ± 65.45	155.00 ± 52.74
Progesterone (nmol/L)	1.08 ± 0.29	1.36 ± 0.68
Testosterone (nmol/L)	1.33 ± 0.60	1.12 ± 0.63
Follicular Stimulating Hormone (IU/L)	85.0 ± 26.9	68.6 ± 28.9
Luteinizing Hormone (IU/L)	38.8 ± 12.3	33.4 ± 11.2
Sex Hormone-Binding Globulin (nmol/L)	45.04 ± 15.96	43.84 ± 21.91
Androgen Index	0.213 ± 0.131	0.146 ± 0.118
Cortisol (nmol/L)	215.58 ± 113.88	190.09 ± 100.50
Insulin (pmol/L)	46.08 ± 20.81	64.90 ± 50.98
Fasting Glucose (nmol/L)	5.33 ± 0.49	5.39 ± 0.80
Total Cholesterol (nmol/L)	5.53 ± 1.32	5.08 ± 1.21
LDL (nmol/L)	3.42 ± 1.15	3.12 ± 0.78
HDL (nmol/L)	1.57 ± 0.35	1.28 ± 0.57
CH/LDL (nmol/L)	3.69 ± 1.16	3.98 ± 1.03
Triglyceride (nmol/L)	1.21 ± 0.61	1.33 ± 0.64

No significant differences were not detected between the two groups, indicating that there are no hormonal variations between these two ethnicities. ·A complete blood sample was not available for 1 participant in the ME group

No significant differences were detected between the two groups FOR_{max} characteristics, indicating that there are no significant variations in FOR or the elicited intensity between these two ethnicities. FOR_{max} values and the respective $\%VO_2peak$ for both trials of the protocol were statistically analyzed. A second trial of the FOR protocol for one participant in the ME group was not obtained due to inability to complete the full protocol and therefore n = 22. The strength of the relationship between the two trials for FOR_{max} is R^2=0.829 (p<0.00) and $\%VO_2peak$ is R^2=0.333 (p=0.13). Since no significant differences were detected from one trial to the next, the 2 trials were then compiled and fitted to a single curve for each participant to allow for more data points to create a best-fit third order polynomial curve (Figure 1).

Figure 1 contains the best-fit third order polynomial FOR curves for the C and ME participants at FOR_{max}, and 5%, 10%, and 20% below the FORmax, along with the respective variance bars for both groups. The FOR is plotted in respect to $\%VO_{2peak}$ (GraphPad PRISM 9). These specific points provide a range of exercise intensities that elicit the highest FOR per group.

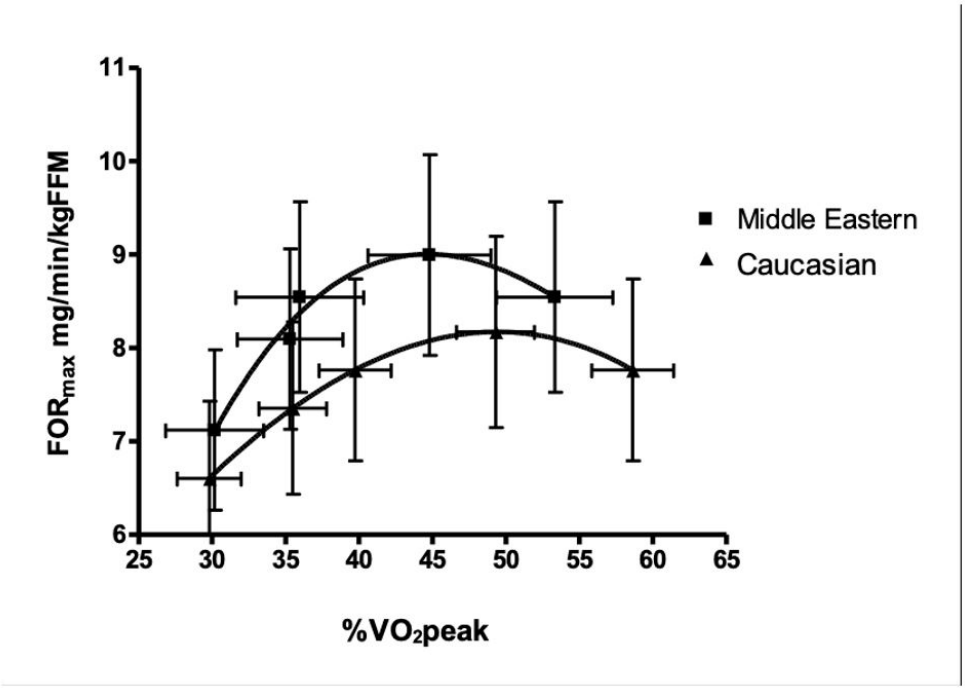

Figure 1. Fat-oxidation rate curves of both participant groups plotted at 5%, 10%, and 20% below FORmax

Discussion

The purpose of this study was to compare FOR at rest and during incremental exercise, FORmax, hormone levels, anthropometric characteristics, PA levels and cardiometabolic risk factors between postmenopausal ME and C women. It was hypothesized that the ME women would exhibit lower FOR at rest and at different sub-maximal exercise intensities and therefore have a lower FOR_{max} as well as, have lower levels of estrogen and possess higher cardiometabolic risk factors compared to the C women. In contrast to the hypothesis, no statistically significant differences were observed between resting FOR, FOR during incremental exercise, FOR_{max}, hormone levels, height, body fat%, skinfold measurements, PA levels, resting BP, resting HR, resting VO_2, exercise HR max, fasting blood glucose and fasting blood lipid profile. The ME women did not exhibit lower FOR at rest, during incremental exercise or at maximum exercise and ME women did not have a lower level of estrogen or have more cardiometabolic risk factors than the C women. As a result of this research, new information was reported on FOR at rest and maximal intensity using treadmill based protocols for ME and C postmenopausal women. The current study is one of the few that utilized a treadmill based protocol to measure and calculate FOR over a wide range of sub-maximal exercise intensities and provide values on FOR and FOR_{max}. PA levels and anthropometrics were also obtained in this study providing detailed data on the health and fitness characteristics of the study populations. Given the lack of statistical significance between the FORs for ME and C post-menopausal women, this study supports the use of an ethnically diverse study participant pool for the purposes of FOR related investigations as well as, help shed light on prevention and treatment techniques for ethnically

diverse participant pools. Associations have been made in a longitudinal cohort study, between elevated levels of testosterone and suppressed levels of sex hormone binding globulin with overall obesity and abdominal obesity in women independent of age, PA and other chronic health conditions [24]. In a cross-sectional study, elevated testosterone and low levels of SHBG were found to impair fasting glucose in postmenopausal women [25]. Therefore, the effects of testosterone on the physiological process within women during the postmenopausal term are amplified.

Limitations

Although the study participants were instructed to maintain regular eating and beverage consumption habits throughout the experiment and to arrive in a fasted state, having only consumed 500ml of water the morning of testing day, this study did not control for eating habits. Although some individuals demonstrated greater intra-variability, no significant differences were observed.

Nutrition plays a vital role in FORs, ingesting carbohydrates in the hours prior to testing decreases the FOR significantly when compared to fasted conditions. Contrary to this, conducting the testing in a fasted stated >6 hours increases FOR [26]. Moreover, FOR have been demonstrated to decrease after consuming a high fat diet, partially due to lower amounts of glycogen storage. When deciding about the rigor of controlling nutrition, there arises a dilemma between internal (as standardized as possible) and external validity (as closely resembling the "real" world as possible) [18]. It was the aim of the present study to employ control measures within the scope of restrictions that can be realistically applied to the general population. This gives an opportunity to define more rigorous procedures to arrive at an improved reliability.

In addition, if one were to perform a power analysis, in order to attain power at the 0.05 level for a two-tailed alpha with an effect size of 0.8, the number of participants in each group would have to be 60. The sample size was slightly underpowered which may have had an influence on the lack of statistical significance in the results.

Conclusions

In summary, this study contributed to the growing body of literature on FOR and women's health by studying an ethnic group that has not been previously investigated. Significant differences were not observed between resting FOR, sub-maximal exercise FOR, FOR_{max}, hormone levels, height, body fat%, skinfold measurements, resting BP, resting HR, resting VO_2, HR max, fasting blood glucose and fasting blood lipid panel. In addition, this study is the first to examine FOR_{max} and measure the hormones that may impact fat mobilization and oxidation during exercise in ME women. The current study is one of the few that utilized a treadmill-based FOR_{max} protocol to measure and calculate FOR over a wide range of sub-maximal exercise intensities and provide values on FORmax. Furthermore, the statistical analysis of the acute day-to-day variability of the treadmill-based protocol for determining FOR_{max}, revealed no significant differences. Given its reliability, the results of the investigation support the use of this modified Achten treadmill protocol for further investigations examining $FOR_{max.}$

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