Short period of exercise causes rapid increase of serum interleukin 6 with no effect on its soluble receptor

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Submitted: 20 February 2009
Accepted: 18 May 2009

Arch Med Sci 2009; 5, 3: 364-370

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Abstract

Introduction: Interleukin 6 (IL-6) is a pleiotropic cytokine secreted by skeletal muscle during exercise, intermediating beneficial effects of physical training such as increasing insulin sensitivity and suppressing pro-inflammatory cytokines. As IL-6 actions depend on its soluble receptor (sIL-6R), the changes in IL-6 concentration should be analyzed together with the changes of the sIL-6R.
Material and methods: We have performed a study to investigate the effects of short term exercise on plasma concentrations of IL-6 and sIL-6R in healthy subjects. Twenty four healthy, non-obese males (age 40-59) were included in the study. A 9 min standard Bruce protocol treadmill stress test was performed. Plasma IL-6 and sIL-6R concentrations were assessed prior to the exercise test and immediately after the 9th min of exercise.
Results: Resting plasma IL-6 and sIL-6R concentrations were 1.54 ±0.43 pg/ml and 40 ±10.5 ng/ml respectively. After exercise IL-6 concentration increased to 2.08 ±0.89 pg/ml ($p < 0.005$ in comparison to baseline). The concentration of sIL-6R was similar to the initial values (39.6 ±7.4 ng/ml). In regression analysis the extent of IL-6 increase during exercise depended mainly on the maximal heart rate and systolic blood pressure. Analysis of ROC revealed that patients with lower maximal heart rate (< 140 bpm) and systolic blood pressure (< 160 mm Hg) were more likely not to have IL-6 rise after exercise.
Conclusions: In healthy males, brief exercise causes significant increase of plasma IL-6 concentration that depends on hemodynamic effects of exercise, whereas the plasma concentration of IL-6 circulating receptor remains stable.

Key words: interleukin 6, IL-6 receptor, exercise, exercise test.

Introduction

The worldwide epidemics of cardiovascular and metabolic diseases turned the attention of the medical community towards the beneficial effects of physical exercise. There is support for the notion that a training programme may decrease concentrations of inflammatory cytokines and improve endothelial function and insulin sensitivity in patients with atherosclerosis and metabolic syndrome [1]. Therefore, there is a large number of studies investigating the mechanisms mediating the beneficial effects of physical training.

One of the most important molecules involved in this phenomenon is interleukin 6 (IL-6), sometimes even called an "exercise factor" [2]. This pleiotropic cytokine used to be considered one of the major villains in cardiovascular pathology. Its high serum concentration is associated with

a higher risk of acute coronary syndromes and worse prognosis in heart failure [3, 4]. Only recently it was pointed out that lack of this cytokine completely abolishes beneficial effects of ischaemic preconditioning [5] and accelerates atherosclerosis in mouse models [6]. It has been shown that IL-6 is secreted by skeletal muscle in response to chronic exercise [7] and may mediate the beneficial effects of exercise on distant organs (liver, brain, etc.) [2].

Equivocal role of IL-6 may be explained to some extent by the interplay of IL-6 with its receptor (IL-6R or gp80) that not only is expressed on the cell membrane, but also has a functional soluble form (called sgp80 or sIL-6R) [8]. This form circulates in the blood and by binding the IL-6 forms complexes that elicit the effects of IL-6 on the cells devoid of the membrane bound IL-6R. Previous studies have shown that the increase of IL-6R expression after strenuous exercise depends on the stimulation by IL-6 [9].

One must remember, however, that effects of exercise induced IL-6 changes (including complex interplay between the IL-6 and its receptor) are not confined to the cardiovascular system. IL-6 released from contracting muscles seems to exert an anti-inflammatory effect [10]. Moreover, it may cross the blood-brain barrier and affect mood and cognition [2, 11]. Nevertheless, some studies reported less enthusiastic results concerning the interplay between the IL-6 and exercise in the heart [12]. Definitely, one of the main recipients of its signalling is the liver, the target organ for all effects concerning glucose or protein metabolism [2].

Although there is evidence about effects of prolonged exercise on IL-6 and sIL-6R [13, 14], the information on the influence of short bouts of exercise is still lacking. Still, daily short episodes of exercise may affect the cardiovascular risk [15]. Therefore we have designed a study to investigate the effects of short bout of exercise on the plasma concentration of IL-6 and its soluble receptors IL-6R.

Material and methods

Participants

The study comprised 24 healthy males (age 40-59, average 50.1). Exclusion criteria were the following: contraindications to treadmill exercise test, known chronic inflammatory or neoplastic diseases, previous history of diabetes or other glucose metabolism disturbances [all patients had an oral glucose tolerance test (OGTT) performed prior to the enrolment], hypertension, symptomatic atherosclerotic artery disease (including ischemic heart disease, peripheral artery disease or stroke), recent infection (within 6 weeks), usage of non-steroidal anti-inflammatory drugs or other anti-inflammatory medications within the last week, obesity. Subjects were not taking any medications on regular basis. To exclude participants with asymptomatic left ventricular systolic dysfunction echocardiography was performed and only subjects with normal left ventricular ejection fraction (> 60%) were included in the study. In all patients fasting insulin concentration was assessed by commercially available immunoenzymatic assay (Medgenix EASIA). Homeostasis model (HOMA) of insulin sensitivity was calculated ($HOMA = Ins_0 \times Glu_0/22.5$).

The study design complied with the Declaration of Helsinki as revised in 1996 and it was approved by the local ethical committee on human research (Institutional Review Board – Local Bioethics Committee of Medical University of Bialystok). All participants gave informed consent prior enrolment to the study.

Exercise and blood sampling

In all subjects a 9 min treadmill exercise test was performed according to Bruce protocol [16]. The tests took place in the early afternoon approximately 4 to 6 h after the morning meal. Nine min in Bruce protocol are considered 10 metabolic equivalents (METs), and approximately is associated with an oxygen consumption of 35 ml/kg [17]. ECG, heart rate and blood pressure were monitored during the stress test. Double product was calculated by multiplying the systolic blood pressure in mm Hg and heart rate in beats per minute. Nobody fulfilled criteria for premature test termination (ECG signs of ischemia, stenocardia, excessive or paradoxical pressure response) [16].

All participants had venous access (22G diameter) inserted by the antecubital vein before the exercise. Blood samples were taken to heparinised syringes (lithium heparin Sarstedt) without using stasis prior to the test and immediately after the 9th min of the exercise. Blood was then centrifuged in 4°C for 7 min in 4000 g, heparinised plasma was collected and frozen in –70°C until further analysis.

Measurements and data analysis

Concentration of IL-6 and sIL-6R were measured using commercially available colorimetric enzyme-linked imunosorbent assays (ELISA) – Quantikine R&D (USA). According to the manufacturer the tests recognize both free cytokines as well as the IL-6-sIL-6R complexes, whereas they cannot distinguish between the forms. Hence, in our study we analyzed total concentrations of IL-6 and sIL-6R (both free and bound in complexes).

Variable distribution was analysed using the Shapiro-Wilk test. Statistical analysis (Statistica 8, StatSoft) was performed using a non parametric test (Mann-Whitney or Wilcoxon when repeated measurements were compared), due to pre-

dominant not normal variable distribution. Spearman's correlation, multivariate regression analysis and receiver operating characteristic curves (ROC) with c-statistics were used to establish the influence of particular variables on the change of IL-6 during the exercise test. For multivariate regression analysis a backward removal method was used. Initial model included all variables that have been previously postulated to be involved in IL-6 secretion: age, BMI, sex, HOMA, insulin and glucose levels, as well as hemodynamic parameters (baseline and maximal pressures and heart rate). Subsequently, Statistica 8.0 software rejected all variables that have been found redundant. This method yielded the best model as assessed by the value of R^2. A p value of less than 0.05 was considered statistically significant.

Results

All subjects had normal enrolment tests: OGTT, echocardiography, and body mass index. General baseline characteristics are given in Table I. Nobody had any stenocardia or ECG changes during exercise test. The exercise caused significant increase in heart rate from 83.3 ±14. to 152.3 ±17.5 beat/min and in systolic blood pressure from 127.8 ±11 to 171.4 ±19.8 mm Hg (p < 0.001 Wilcoxon's test) whereas the diastolic blood pressure changed only from 85.4 ±9.11 to 89.4 ±12.6 mm Hg (p = 0.2). The attained heart rate was on average 89.7 ±11% of the approximate maximal heart rate The calculated maximal double product averaged 26.34 ±5.74 mHg × beats/min.

This effort caused an increase of the plasma IL-6 concentration from 1.54 ±0.43 to 2.08 ±0.89 pg/ml (p < 0.005 Wilcoxon test) (Figure 1A). Simultaneously, no significant change of the concentration of soluble IL-6 receptor was found (40 ±10.5 ng/ml in sedentary vs. 39.6 ±7.4 ng/ml after exercise) (Figure 2A).

The increase of the IL-6 concentration after the exercise test correlated with the haemodynamic consequences of exercise: maximal heart rate, maximal systolic blood pressure, and double product (Table II). There were no statistically significant correlations between insulin sensitivity index (HOMA), fasting glucose, fasting insulin levels, age, BMI and IL-6 level changes during exercise. Multivariate regression analysis provided evidence that age, BMI, fasting insulin concentration, maximal heart rate during exercise, and maximal systolic blood pressure during the test independently determine the increase of IL-6 during exercise test. Among abovementioned factors, age and fasting serum insulin concentration presented negative association with the increase of IL-6 level (Table III).

We divided our study population into two groups based on the presence of increased IL-6 level after the exercise test. In 17 out of 24 patients the concentration of IL-6 after the exercise test exceeded 110% of the baseline value. These patients formed the first group (Table I). The second one (n = 7) was composed of patients who did not present an increase of IL-6 during exercise. The patients in the first group had a significantly higher

Table I. General characteristics of the study population

Variable	All subjects (n = 24)	IL-6 increase (n = 17)	No IL-6 increase (n = 7)
Age [years]	50.1 ±5.5	51 ±5.4	48.7 ±6
Current smokers	5 (20%)	5 (29%)	0
LV ejection fraction	63.1 ±3	63.1 ±3.2	62.9 ±2.7
BMI	25.1 ±2.7	24.5 ±2.7	26 ±2.7
Waist circumference [cm]	91.8 ±9	92 ±6.4	92.9 ± 9.1
Fasting glucose concentration [mmol/l]	5.1 ±0.4	5.1 ±0.5	5.1 ±0.3
Fasting insulin concentration [pmol/l]	92.3 ±26.3	88.7 ±18.6	101 ±40
IL-6 concentration before exercise test [pg/ml]	1.54 ±0.43	1.52 ±0.48	1.57 ±0.29
IL-6 concentration after exercise test [pg/ml]	2.08 ±0.89	2.35 ±0.91	1.44 ±0.44*
sIL-6R concentration before exercise test [ng/ml]	40 ±10.5	37.8 ±6.1	45.1 ±16.7
sIL-6R concentration after exercise test [ng/ml]	39.6 ±7.4	38.7 ±6.2	41.9 ±16.1
Maximal heart rate during exercise test [beats/min]	153.7 ±18.4	160 ±16.5	138 ±13.4*
Maximal systolic blood pressure during exercise test [mm Hg]	172.1 ±19.8	178.5 ±19.7	156.4 ±8**
Maximal double product [mHg × beats/min]	26.34 ±5.74	28.7 ±5.4	27.1 ±3.1**

Values are given as mean ± standard deviation

**p < 0.05, **p < 0.005 vs. group with IL-6 increase after exercise test, Mann-Whitney test*

maximal heart rate, maximal systolic blood pressure and double product than the ones in the second cohort (Table I). Analysis of ROC curves proved that the same variables had statistical power (c-statistics from 0.845 to 0.887) to identify patients in whom the rise of IL-6 during exercise did not occur (Table IV). Patients, in whom the 9 min exercise test according to the Bruce protocol did not cause heart rate greater than 140 beats/min or systolic blood pressure higher than 160 mm Hg usually did not have significant increase of serum IL-6 concentration (Table IV).

Discussion

In this study we were able to show that even a short lasting exercise may increase plasma concentration of IL-6 in healthy subjects, provided there is sufficient hemodynamic effect. Simultaneously, this effort did not affect concentration of sIL-6R. These results supplement previous findings that prolonged exercise causes expression of IL-6 in skeletal myocytes that ensues in its secretion to the circulation [18]. Our study, unlike the previous ones, used a short submaximal exercise and the blood sampling was performed in short time intervals, which suggests that the observed findings should not be attributed to the *de novo* production of IL-6 protein, but rather secretion of the pre-formed cytokine. Previous studies where *de-novo* mRNA expression in muscles was investigated presented significant increase first after 90 min after onset of the exercise [18], whereas we have observed an increase already after 9 min of exercise. This model, unlike the previously published ones, resembles short bouts of exercise present in regular every day activity and does not aspire to the investigation of the effects of prolonged physical training.

We were able to show not only the very early increase of IL-6 concentration during exercise, but also point out that this rise strongly depends on the hemodynamic consequences of the work-out. All participants were subjected to the same intensity of exercise (9 min of Bruce protocol exercise test), but

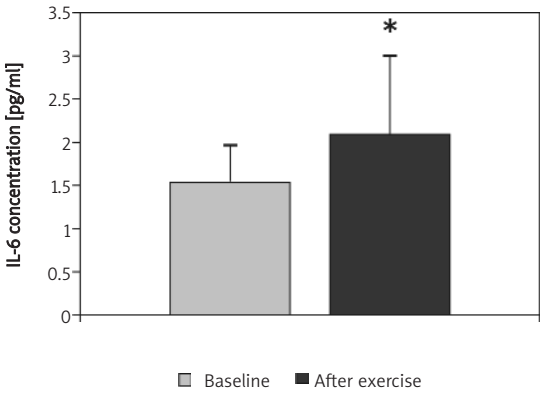

Figure 1A. Average concentration of interleukin 6 (± standard deviation) in heparinised plasma in sedentary subjects (baseline) and after 9 min of exercise (after exercise). Exercise caused significant increase of the IL-6 concentration

**p < 0.005 Wilcoxon test vs. baseline*

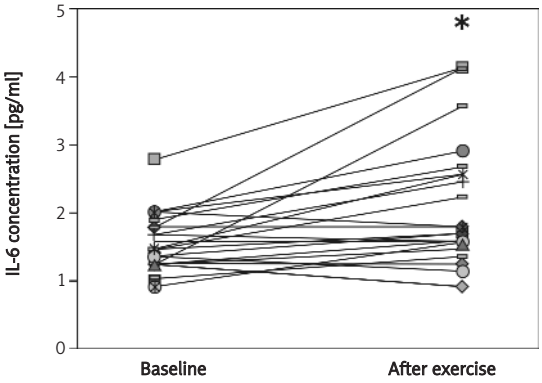

Figure 1B. Plasma concentration of interleukin 6 in individual participants prior (Baseline) and after the exercise test

**p < 0.005 Wilcoxon t test vs. baseline*

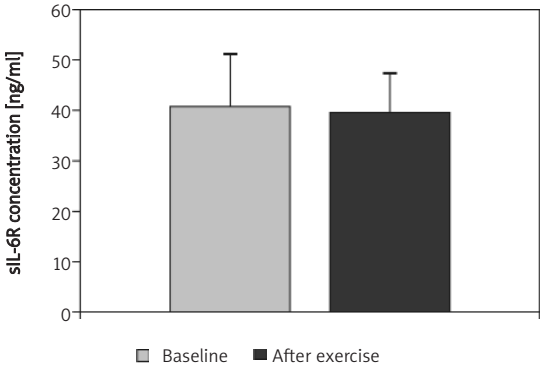

Figure 2A. Average concentration of soluble interleukin 6 receptor (± standard deviation) in heparinised plasma in sedentary subjects (baseline) and after 9 min of exercise (after exercise)

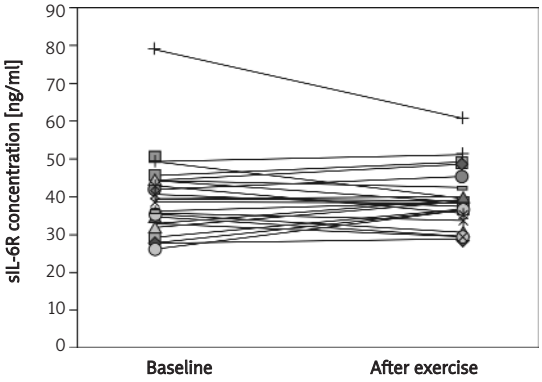

Figure 2B. Plasma concentration of soluble interleukin 6 receptor in individual participants prior (baseline) and after the exercise test

the same bearing may have different effects on particular persons. Our analysis has shown that most subjects who during exercise do not reach a heart rate of 140 beats/min or systolic blood pressure of 160 mm Hg do not have an increase of IL-6 concentration. This finding is in concert with previous papers suggesting de novo IL-6 secretion to be effort dependent [7]. Nevertheless, our report extends this finding to the early IL-6 release that, probably due to other time frame, depends on a different mechanism and unlike the chronic one does not involve *de-novo* synthesis of protein [18]. We have observed an influence of BMI, age and insulin concentration on the exercise induced IL-6 rise. These parameters are strongly associated one with another and with IL-6 [2, 15]. The effect of age may be explained on the basis of different hemodynamic responses in older patients (decreasing maximal heart rate with age [17]). On the other hand increased fasting insulin concentration may identify patients who have attenuated IL-6 response to exercise.

Previous studies presented evidence that strenuous, long lasting exercise may increase both cellular expression of IL-6R [9] and serum concentration of its soluble form [14]. In the former paper IL-6 has been shown to be the main regulatory mechanism triggering expression of IL-6R. We were not able to confirm this phenomenon in our setting. Nevertheless, our data complement rather than contradict previous reports suggesting that a short exercise may cause early secretion of IL-6 and in this way regulate expression of IL-6R in longer term. The changes in plasma concentration of sIL-6R do not occur after short lasting exercise probably because translation and transcription processes are necessary. Lack of repeated blood samplings following the end of the exercise is one of the limitations of our study. It is conceivable that an increase of sIL-6R concentration might follow within 2 or 3 h after the end of exercise. However, a very recent paper [13] presented evidence that even after much longer exercise the rise in the sIL-6R may be expected first on the second day after the exercise.

IL-6 is prototypical pleiotropic cytokine involved in various physiological processes from inflammatory reaction [19], regulation of apoptosis [20] and to bone turnover [21]. In order to exert an effect on a cell IL-6 has to be bound first by gp80 and subsequently this complex binds the gp130. It is

Table II. Variables significantly correlated with change of IL-6 concentration during exercise test in Spearman's test

Variable	*r*	*p*
Maximal heart rate during exercise test [beats/min]	0.55	0.006
Maximal systolic blood pressure during exercise test [mm Hg]	0.55	0.005
Maximal double product [mHg × beats/min]	0.59	0.002

Table III. Variables independently and significantly associated with the change of IL-6 concentration during exercise test. Multivariate regression analysis. Characteristics of the model: $R^2 = 0.6162$, $F(6,17) = 4.5492$, $p < 0.0063$

Variable	B(±SD)	*p*
Age [years]	–0.044 ±0.02	0.048
BMI	0.16 ±0.05	0.008
Fasting insulin [pmol/l]	–0.016 ±0.006	0.013
Maximal heart rate during exercise [beats/min]	0.11 ±0.048	0.033
Maximal systolic pressure during exercise [beats/min]	0.11 ±0.046	0.029

Table IV. Analysis of ROC curves for variables that may identify subjects in whom short physical exercise does not cause increase of serum IL-6 concentration

Variable	C - statistics	CI	Proposed cut-off value	Sensitivity	Specificity
Fasting insulin concentration [pmol/l]	0.517	0.199-0.835	> 125	0.421	0.941
Maximal heart rate during exercise test [beats/min]	0.845*	0.673-1	< 140	0.714	0.882
Maximal systolic blood pressure during exercise test [mm Hg]	0.887*	0.748-1	< 160	0.857	0.824
Maximal double product [mHg × beats/min]	0.866*	0.714-1	< 21	0.714	0.941

*$p < 0.05$

gp130 that starts the intracellular transduction cascades: JAK-STAT, PI3K-Akt and MEK MAPK [8]. Thus, IL-6 requires an abundance of both its receptors in order to have a biological effect. Although gp130 is ubiquitously expressed on almost all cell types, gp80 (IL-6R) is absent in many tissues. However, it has been described that complexes of IL-6 with a circulating form of gp80 – sgp80 bind the gp130 on the cell surface, starting the intracellular cascade typical for IL-6 stimulation on cells that do not express gp80 subunit [8]. Therefore, in order to understand fully the effects of IL-6 concentration changes they should be analysed simultaneously with the sIL-6R levels.

There is a plethora of apparently contradictory reports describing the role of IL-6 in the cardiovascular system. Epidemiological studies reported a strong association between high IL-6 levels and poor prognosis [4]. On the other hand, precisely designed experimental studies performed both on humans and laboratory animals provided evidence for protective effects of IL-6 [5, 6, 18]. Recent reports suggest that there might be a distinctly different consequence of chronically elevated IL-6 concentrations (e.g. in heart failure or in metabolic syndrome) and short "impulses" of IL-6 induced by exercise or ischemic preconditioning [7, 10]. On one hand, briefly increased IL-6 abundance may cause activation of protective intracellular transduction pathways, on the other, prolonged elevation of IL-6 concentration may lead to desensitization hampering of the cytoprotective response as it has been described in other cytokine systems [22].

The pleiotropic effects of IL-6 may, to some extent, resemble the effects of exercise [7]. Physical training is currently considered a key element in both primary and secondary prevention of cardiovascular diseases [23]. It may affect the prognosis of patients in various ways. Exercise improves endothelial function [1], diminishes concentration of inflammatory markers [1], and prevents development of metabolic syndrome [24] probably by improving insulin sensitivity [15]. Moreover, a study by Laufs *et al*. provided evidence that exercise may cause an increase in the number of circulating endothelial progenitor cells (EPC) and angiogenic factors [25]. According to meta-analysis by Smart *et al.* [26], physical training significantly decreases mortality and morbidity in patients with ischemic heart disease or heart failure. Interestingly, the benefits of exercise are not restricted to the participants of special training programs. It should be stressed that even a higher everyday physical activity diminishes cardiovascular risk and improves insulin sensitivity [15]. These results suggest that there must be a beneficial effect of very short periods of moderate exercise. Hence, there is a need for further research in this model that has been neglected in recent years. It is of particular interest because even short lasting exercise has been proven to exert cytoprotective effects on ischemic organs (heart and skeletal muscles) [27]. It should be established in future studies whether IL-6 may be involved in this pheneomenon as it is in ischemic precondiotioning [5].

The study has several limitations. We did not measure the arterio-venous difference, therefore we can only indirectly suggest the increase of IL-6 secretion by skeletal muscles based on the extrapolation of the previous studies [18, 28]. Nevertheless, increased plasma concentration of IL-6 may be the result of decreased cleavage or decreased cell membrane receptor binding [29]. The latter option is less likely because we did not observe any significant changes in sIL-6R concentration. We may not exclude other sources of IL-6 than skeletal muscle. Since this cytokine may be produced by endothelial cells, vascular smooth muscle cells and cardiomyocytes [8], hemodynamic changes may affect all those cell types and might cause IL-6 secretion. This hypothesis requires further investigation.

There are new questions emerging after the results of our study. One of them is why IL-6 rise depends on the blood pressure and heart rate. We presented an association that does not exclude either the influence of blood pressure on IL-6 release or the effect of IL-6 on haemodynamics. Nevertheless, the connection between IL-6 and hemodynamic parameters during exercise on one hand confirms previous results by the group of Pedersen [7, 9, 18, 28], on the other extends their findings to short lasting exercise [28]. Therefore, our results may have important clinical and social implications. Even short bouts of physical exercise may have beneficial effects if they cause a sufficient increase in heart rate and systolic blood pressure. This hypothesis should be verified in a large prospective clinical trial.

Our study gives a basis for a physiological connection between short bouts of exercise in everyday activity and possible favourable cardiovascular effects. The link may involve short impulses of IL-6 secreted following brief vigorous exercise similar to the one in our study. This may be a similar phenomenon as the one previously described by Pedersen *et al*. who have shown that an increase of IL-6 concentration following strenuous exercise may improve insulin sensitivity [7].

In conclusion, a short bout of exercise causes a rapid and significant increase of the plasma IL-6 concentration in healthy subjects. The time of this rise suggests secretion of the pre-formed IL-6 from muscles. Brief exercise does not affect the plasma concentration of the soluble IL-6 receptor (sIL-6R) in the short term.

Acknowledgments

The project was supported by grant from the Medical University of Bialystok 3-53828.

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