Energy expenditure, sex, and endogenous fuel availability in humans

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Adipose tissue lipolysis supplies circulating FFAs, which largely meet lipid fuel needs; however, excess FFAs, can contribute to the adverse health consequences of obesity. Because “normal” FFA release has not been well defined, average (mean of 4 days) basal FFA release and its potential regulation factors were measured in 50 lean and obese adults (25 women). Resting energy expenditure (REE), but not body composition, predicted most of the interindividual variation in FFA release. There was a significant, positive linear relationship between palmitate release and REE; however, women released approximately 40% more FFA than men relative to REE. Neither plasma palmitate concentrations nor respiratory quotient by indirect calorimetry differed between men and women. Glucose release rates were not different in men and women whether related to REE or fat free mass. These findings indicate that nonoxidative FFA clearance is greater in women than in men. This could be an advantage at times of increased fuel needs. We conclude that “normal” adipose tissue lipolysis is different in men and women and that the fuel export role of adipose tissue in obesity will need to be reassessed.


Introduction
Approximately 50% of postabsorptive energy needs in humans are supplied from the oxidation of fatty acids. The major source of this lipid fuel is circulating FFAs that are released from lipolysis of adipose tissue triglyceride. Maintaining normal FFA availability is of considerable importance in human health (1), because high FFA concentrations are associated with a number of cardiovascular risk factors (2) and a predisposition to type 2 diabetes mellitus (3). Experimentally increasing FFA concentrations in normal humans can induce insulin resistance (4), disordered lipoprotein metabolism (5), altered vascular reactivity (6), and abnormal insulin secretion (7).

At rest, an average adult with 15 kg of body fat (>50 moles of fatty acids) releases less than 0.4 mmol/min into the circulation to provide energy for fat-free tissue. This is in striking contrast to glucose fuel, where hepatic glycogen stores are approximately 370 mmol and glucose is released at rates of approximately 0.8 mmol/min (8). Understanding the regulation of this relatively massive adipose tissue fuel depot is important given the prevalence of obesity is increasing rapidly in many Western societies.

The factors that regulate changes in FFA release in response to changing lipid fuel needs are relatively well described. The insulin secretion stimulated by carbohydrate ingestion markedly inhibits FFA release, thereby suppressing FFA concentrations and fatty acid oxidation by 80–90% (9). In contrast, carbohydrate deprivation or fasting reduces insulin secretion, which enhances adipose tissue FFA release, allowing FFA concentrations to increase by approximately 300% to facilitate greater fat oxidation. Exercise, the most potent physiological stimulus to fat oxidation, can increase FFA release by more than 300% (10), primarily through increased catecholamine stimulation of adipose β-adrenergic receptors. This relatively good understanding of the short-term regulators of FFA release is not mirrored by an equal appreciation of the factors that influence average resting FFA availability.

Understanding the normal interaction between adipose tissue fuel release rates and fat-free mass (FFM) fuel use rates is important for understanding the effects of body fat on health. The approach to comparing fuel release rates between groups with different weights and/or different body composition can influence how the information is interpreted, however. For example, there has been controversy regarding whether obesity causes higher FFA concentrations because of greater release or lesser clearance of FFA from the circulation. Obese adults have FFA release rates similar to lean adults, relative to body weight or body fat (11, 12),
Included within the obese group.

Men and women were lean and one-half were obese were recruited such that approximately one-half of the functions. All women were premenopausal. Subjects blood pressure, hematological indices, liver and renal acceptance test prior to entering the study and had normal previous 3 months. All had a normal oral glucose tol-

taking medications, and had been weight stable for the Board–approved study after written, informed consent

women) participated in this Institutional Review

Methods

Subjects. Fifty research volunteers (25 men and 25 women) participated in this Institutional Review Board–approved study after written, informed consent was obtained. All participants were nonsmokers, not taking medications, and had been weight stable for the previous 3 months. All had a normal oral glucose tolerance test prior to entering the study and had normal blood pressure, hematological indices, liver and renal function. All women were premenopausal. Subjects were recruited such that approximately one-half of the men and women were lean and one-half were obese and that a wide range of body fat distribution was included within the obese group.

Experimental design. Because body composition is a potentially important determinant of fuel metabolism, it was assessed in several ways. These included the fol-

lowing: (a) dual-energy x-ray absorptiometry (Lunar Radiation Corp., Madison, Wisconsin, USA) to measure total body fat and FFM (21); (b) a single-slice computed tomography (CT) scan (GE-Imatron Inc., San Francisco, California, USA) of the abdomen at the image level (22) to assess central fat accumulation (intra-

abdominal and abdominal subcutaneous fat); (c) the waist-to-hip circumference ratio (WHR) to evaluate relative body fat distribution; (d) needle-liposuction adipose tissue biopsies were performed to determine abdominal subcutaneous and gluteal fat cell size. Procedures for the measurement of fat cell size were fol-

wed according to the methods of Di Girolamo et al. (23). At least 200 cells per site were sized to provide an estimate of average fat cell diameter. Average fat cell diameter was then used to calculate fat cell volume and lipid content (23). BMI was calculated as body weight (kilograms) per height (meters squared).

All participants were provided weight-maintaining meals (40% carbohydrates, 40% fat, 20% protein) in the Mayo Clinic General Clinical Research Center (GCRC) for 2 weeks prior to the studies, including the 4 study days. At the end of the 2-week period participants were admitted to the GCRC for four consecutive overnight stays. During the study days the volunteers were allowed to perform their usual activities (including work), but received their meals in the GCRC, including the last meal, which was given at 1800 hours, and a snack that was provided at 2100 hours. REE was measured by indirect calorimetry (DeltaTrac Metabolic Cart; Sensormedics Inc., Yorba Linda, California, USA) each morning before the participants arose from bed. The metabolic cart was calibrated each morning prior to the study, and additional quality control for the carts included monthly pressure calibrations and gas cali-

brations together with biannual calibrations of the metabolic carts using an alcohol burn test.

Samples for the measurements of postabsorptive steady-state palmitate and glucose release rates and plasma hormone and catecholamine concentrations (insulin, growth hormone, epinephrine, and norepi-

nephrine) were obtained between 0700 and 0730 hours. The FFA and glucose release rates into the cir-

uculation were measured using continuous intravenous infusions of isotopically labeled FFA ([U-13C]palmitate) (24) and glucose ([6-2H2]glucose) (25), respectively. The needed preservative was not added to the cate-

cholamine collection tubes for three volunteers’ studies, and thus no measures of epinephrine or norepi-

nephrine were available for them. The CT image from one volunteer was unable to be retrieved for analysis.

Four studies were performed because of the need to more accurately define average, resting FFA release given the substantial (coefficient of variation of ~30%) day-to-day intraindividual variability in resting FFA kinetics (26). By more accurately defining the relation-

ship between average FFA availability and REE (or body composition) for each participant we improved the ability to test for other variables (such as body fat or
plasma hormone concentrations) that might also influence FFA release rates.

Palmitate was chosen as a representative fatty acid to allow assessment of FFA kinetics because it is one of the major FFAs (29% ± 3% in these volunteers) and because its metabolic properties are representative of the remaining seven major FFAs (26). Palmitate was the same proportion of FFA in men and women. Because circulating glucose kinetics are less variable from day to day (27), they were measured on 2 of the 4 days.

**Analysis of samples.** Plasma palmitate enrichment was measured using gas chromatography/combustion/isotope-ratio mass spectrometry as described (24). Plasma glucose enrichment was measured using gas chromatography/mass spectrometry (25). Insulin and growth hormone concentrations were measured using chemiluminescent sandwich assays (Sanofi Diagnostics Pasteur Inc., Chaska, Minnesota, USA), and catecholamines were measured using HPLC with electrochemical detection (28).

**Calculations.** Steady-state appearance (and disappearance) rates of palmitate and glucose were calculated using the mean enrichment values and tracer infusion rates as described previously (24, 25).

**Statistical analysis.** The average palmitate release rate (micromoles per minute) was used as the dependent variable in a multivariate, stepwise regression analysis using the following candidate independent variables: average REE, sex, FFM, fat mass, percentage of fat, intra-abdominal fat area, abdominal fat cell size, and average insulin, growth hormone, and catecholamine concentrations. Variables that were not normally distributed (such as plasma epinephrine concentrations) were log transformed in order to be appropriately tested for inclusion in the model. Using the approach of stepwise variable selection, stepping up, only variables with a significance level of 0.05 were included in the model. (Using a looser criterion of $P < 0.10$, the model was the same.) We also tested quadratic terms and two-way interactions among variables in the final model and found none significant. To ensure that FFM and total fat mass were not prematurely excluded from consideration, they were included in a non-stepwise model that also included the variables found to be significant in the stepwise model. Exploratory univariate regression analyses were performed for the purposes of comparing the relative strengths of associations between palmitate release rates and alternative normalizing variables and for the purposes of displaying factors that could explain the residual variance in palmitate rates. Glucose release rates (milligrams per minute) were examined using linear regression analysis versus REE and FFM, with an additional multivariate analysis to test for a sex effect. Likewise, multivariate regression analysis was used to determine whether there was a sex effect on the relationship between FFM and REE.

**Results**

**Subject characteristics.** The characteristics of the subjects are provided in Table 1. The expected differences between men and women in body composition, body fat distribution, and REE were observed. Plasma palmitate concentrations were not different, and with the exception of the expected greater concentrations of growth hormone in women than in men ($P < 0.005$), plasma hormone and catecholamine concentrations were not different. The relationship between REE and

**Figure 1**

REE is plotted vs. FFM for the men and women participating in the study.
FFM was assessed using multiple linear regression analysis using a sex and a sex \times FFM interaction term. This relationship was not different between men and women (Figure 1; simple $r^2 = 0.69$, $P < 0.0001$).

**Day-to-day variability of indirect calorimetry and substrate measurements.** The coefficient of variation (CV) for REE was 4% ± 2% and for the respiratory quotient (RQ) was 3% ± 1%. Perhaps thanks to the greater dietary control imposed by the study design, the CVs for average fasting plasma palmitate concentrations and flux were 16% ± 8% and 14% ± 8% (which is approximately one-half of what we have observed previously) (26). The CV for plasma glucose concentrations over the 4 days was 3% ± 1%, whereas the difference between glucose flux between the 2 study days for which it was measured averaged 13% ± 13%.

**Relationship between FFA release and REE versus FFM.** For the entire group, palmitate release rates were positively correlated with REE ($r = 0.54$, $P < 0.0001$), but were not significantly correlated with FFM (see inset of Figure 2; $r = 0.25$, $P = 0.09$). Because the multivariate regression analysis (see below) indicated a significant sex effect, palmitate release was plotted in relationship to REE separately for men and women (Figure 2) and sex-specific univariate regression analyses were performed. The relationship between palmitate kinetics and REE was stronger for each sex than for the combined group. Palmitate release rates relative to REE were significantly greater in women than in men, such that for a given level of energy expenditure, the average fuel availability from FFA was approximately 40% more in women. Note that the greater FFA release rates were not associated with higher plasma FFA (palmitate) concentrations in women (Table 1). Nor were these sex differences in FFA release associated with differences in resting fuel use; the RQ, which provides a measure of the proportion of fat and carbohydrate being oxidized, was the same in men and women (Table 1).

The initial multivariate stepwise regression analysis results disclosed that REE, sex, and plasma epinephrine concentrations were significant predictors of average palmitate flux. The parameter estimates for the model are provided in Table 2. The $r^2$ for this model was 0.57. Percentage of body fat and indices of central fat distribution (visceral fat area and WHR) were not included in the final model.

To ensure variables were not inappropriately left out of the model by the stepwise regression analysis approach we performed regression analysis using a nonstepping model that included REE, sex, FFM, fat mass, and our measures of adrenergic tone (plasma epinephrine and norepinephrine concentrations) as independent variables. The results were the same as for the stepwise model. The $r^2$ for this model was 0.61. The parameter estimates and associated $P$ values for this model are provided in Table 3. Consistent with the stepwise approach, REE, sex, and epinephrine significantly contributed to the ability to predict the interindividual differences in palmitate flux. Total fat mass, FFM, and plasma norepinephrine did not contribute significantly to this model. Although the parameter estimate for REE appears small, the REE values are expressed in the table per 100 kcal/day (range of values 1,190–2,238 kcal/day), creating a numerically small parameter estimate that was highly statistically significant. The parameter estimate for male sex was −31, consistent with the visual separation of the regression lines for men and women depicted in Figure 2. The parameter estimate for plasma epinephrine should be interpreted in the context of the need to log transform the plasma epinephrine concentrations because of their skewed distribution. Despite the good relationship between REE and palmitate release, there remained individual variation from the sex-specific group relationships (Figure 2). Because the multivariate regression analysis indicated that epinephrine contributed to the interindividual differences in palmitate release rates relative to REE, we examined this interaction in a different manner to allow a better visualization of the interactions. To do this the residual variance in palmitate release was determined for each participant. This value represents how each participant’s average palmitate release rate differed from their sex-specific group and is calculated by

![Figure 2](image-url)

*Figure 2*

Resting postabsorptive palmitate release rate is plotted vs. REE for the men and women participating in the study. The inset depicts the relationship between palmitate release and FFM for these same volunteers.
subtracing the predicted palmitate release (based on
REE and sex-specific regression formulas) from the
observed palmitate release for each subject.
Consistent with the multivariate regression analy-
sis, the mean fasting plasma epinephrine concentra-
tions were positively correlated ($r = 0.33, p < 0.05$)
with residual palmitate release. Although plasma nor-
epinephrine concentrations were not a significant
correlator to the model, they were also positively
correlated ($r = 0.31, p < 0.05$) with residual palmitate
release. There was no significant correlation between
plasma epinephrine and norepinephrine concentrations.
Neither the mean fasting plasma insulin or
growth hormone concentrations were correlated
with the residual variance in palmitate release.
Although indices of body fatness were not significant
predictors of palmitate flux in the regression models,
we have found previously that upper-body obesity is asso-
ciated with elevated FFA concentrations and flux (13).
We therefore explored whether visceral fat area might be
associated with the residual variance in palmitate flux.
In this analysis the residual variance in palmitate release
was correlated ($r = 0.44, p < 0.03$) with visceral fat area
in men (Figure 3). Percentage of body fat and WHR
tended ($p = 0.06$ and $p = 0.07$, respectively) to be posi-
tively correlated with residual variance in palmitate release in men. To our surprise, abdominal fat cell size
did not significantly correlate ($r = 0.24, p = 0.24$) with
residual palmitate flux in men. None of the body fat
parameters (percentage of body fat, kilograms of body
fat, WHR, visceral or abdominal subcutaneous fat area,
or subcutaneous fat cell size) correlated with residual palmitate release rates in women.
If REE is important for determining what constitutes
“normal” FFA release rates, we anticipated that plasma
palmitate concentrations would vary proportionately
with the residual variance in palmitate release relative
to REE. Indeed, there was a strong, positive correla-
tion between residual palmitate release and palmitate
concentrations (Figure 4). Thus, individuals in whom
palmitate release rates were greater than their sex-spe-
cific group relationship had higher plasma concentra-
tions and vice versa.

### Table 3

Parameter estimates for nonstepped multivariate regression analysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter estimate</th>
<th>Standard error</th>
<th>$P$ value</th>
</tr>
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<tr>
<td>Intercept</td>
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<tr>
<td>REE</td>
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<td>2.1</td>
<td>0.0024</td>
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<td>Male</td>
<td>-31</td>
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<td>FFM (kg)</td>
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<td>0.650</td>
<td>0.5507</td>
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<tr>
<td>Fat mass (kg)</td>
<td>0.366</td>
<td>0.316</td>
<td>0.2539</td>
</tr>
<tr>
<td>Plasma epinephrine (log)</td>
<td>16</td>
<td>7</td>
<td>0.0275</td>
</tr>
<tr>
<td>Plasma norepinephrine</td>
<td>0.15</td>
<td>0.12</td>
<td>0.2290</td>
</tr>
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</table>

The parameter estimates for the formula describing the relationship between
palmitate flux (µmol/min – dependent variable) and the following independent
variables: REE (given as per 100 kcal/day); male sex; FFM; fat mass; log
plasma epinephrine concentration; plasma norepinephrine concentration.

Glucose appearance rates. The sex difference in the rela-
tionship between fuel mobilization and REE was spe-
cific to FFA. Glucose release rates correlated directly
with both FFM ($r = 0.71, p < 0.00001$) and REE
($r = 0.62, p < 0.0001$). Multivariate regression analysis
failed to disclose a sex effect on the relationship
between glucose release rates and FFM or REE (Figure 5).
The relationship between glucose appearance and
REE in women ($r = 0.50$) was glucose flux (mg/min) =
$70 + [REE (kcal/day) \times 0.034]$; in men ($r = 0.34$) was
glucose flux $= 82 + [REE (kcal/day) \times 0.039]$. The 95%
confidence intervals for both the intercept and the
slope overlapped for men and women.

### Discussion

These studies were conducted to address two previously
unsettled issues with respect to the relationship
between adipose tissue fuel release and lean tissue fuel
use. Our first concern was that the traditional means
of comparing resting FFA release rates between groups
— dividing by FFM — is inappropriate and misleading.
The second major concern was the lack of information
as to whether men and women could be compared
using the same approach. In addition, by more accu-
ately determining the average basal FFA release rate for
each individual, we were able to examine additional fac-
tors that might relate to basal FFA release. Consistent
with our hypothesis, REE was found to be a better pre-
dictor of basal palmitate release than FFM. Our inabil-
ity to detect an association with FFM after adjusting
for other variables could be because any independent
relationship between FFA release and FFM was too
slight to detect, rather than because there is no rela-
tionship at all. Our second concern, that comparing
men and women directly could lead to problems in
identifying “abnormal” FFA release, was also con-
firmed. The greater relative FFA release rates in women
despite similar plasma concentrations can only be

![Figure 3](image-url)

The relationship between visceral fat area and residual differences
between sex-specific REE/palmitate release (flux) rate relationships
for men and women are shown. In men visceral fat area showed a sig-
nificant, positive relationship ($r = 0.45, p < 0.05$) with the residual
palmitate flux values. In women visceral fat area was not correlated
with the residual palmitate flux values; neither were percentage
of body fat, WHR, or fat cell size.
explained by greater clearance rates. Thus, understanding adipose tissue lipolysis will require that energy expenditure and sex, as well as body composition, be taken into account.

Despite the sex differences in FFA release and uptake there was no difference between men and women in FFA concentrations or in the proportion of energy expenditure accounted for by fatty acid oxidation. This is possible because not all FFAs removed from the circulation are immediately oxidized — significant proportions are re-esterified as tissue triglyceride (29). It follows that at rest women shunt more circulating FFA into re-esterification pathways than do men. Recent studies (30) have shown that lean women have almost double the VLDL-triglyceride production rates as lean men, despite comparable the VLDL-triglyceride concentrations. Thus, greater hepatic clearance, re-esterification, and export of the “excess” FFA back to adipose tissue could explain our findings.

This basic, physiological difference in FFA metabolism between men and women could be advantageous at times of increased energy requirements, such as exercise or stress. For example, adipose tissue lipolysis increases somewhat gradually in response to exercise, whereas energy expenditure and fat oxidation increase almost immediately (10, 31). Women should, therefore, be able to increase the proportion of FFA directed toward oxidation (with a concomitant reduction in FFA re-esterification) and better match circulating lipid fuel availability to lipid fuel needs as energy expenditure increases. The combination of greater availability and greater clearance may explain the ability of women to oxidize relatively more fat than do men during exercise (32). This greater resting lipid fuel release rate is accomplished without exposing tissues to higher and potentially harmful FFA concentrations.

Excess accumulation of central fat is associated with adverse health consequences (33), which have been linked to higher FFA concentrations (1). In this study, indices of body fatness did not contribute significantly to FFA release rates after REE, sex, and epinephrine were considered. The number of individuals with upper-body obesity was small, however, compared with our previous studies (13). As a way to assess whether the results of the present study were in conflict with previous studies we examined whether visceral fat area was associated with greater palmitate release rates after interindividual differences in REE were taken into account. There was an association with visceral adiposity in men, but we did not detect such an effect in women. We reported previously that FFA flux is increased in upper-body obesity when larger numbers of lean and obese women (matched for FFM and REE) were evaluated (13). Therefore, we do not believe that the current data should be taken to indicate that centrally obese adults have the same rates of lipolysis as do lean adults. The narrower range of visceral fat area in women compared with men in the present study (Table 1) may have limited our ability to detect an effect of visceral fat on FFA kinetics.

One cannot infer causal mechanisms from statistical analysis alone: that interindividual variances in REE drive FFA release rates. It might be argued that differences in FFA release rates determine REE. A number of observations lead us to doubt that FFA modulates REE, however. For example, experimental increases in plasma FFA do not raise REE (34). In addition, REE is not reduced by suppression of FFA release and fatty acid oxidation using acipimox (35) or insulin (36), or by pharmacological blockade of fatty acid oxidation (37). We conclude differences in REE seem to be the major, underlying factor influencing the rate at which adipose tissue releases its fuel into the circulation in resting humans. Of note, the intercept of the relationship between REE and FFA release rates was not zero. This indicates that dividing FFA release rates by a measure of energy expenditure is inappropriate. Regression analysis will be needed to compare groups with different mean REE values.

It is not known how REE influences adipose tissue fuel export function in the basal state and why this

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Figure 4
The relationship between plasma palmitate concentration and residual differences between sex-specific REE/palmitate release rate relationships for all volunteers.
relationship is different in men and women. However, interpreting the present data within the framework of previously published studies, we would like to suggest a new concept in the regulation of adipose tissue lipolysis. Plasma FFA concentrations may themselves serve as a signal for the secretion of hormones that modulate lipolytic activity. Insulin, catecholamines, and growth hormone exert rapid effects on adipose tissue lipolysis and are the major regulators of systemic FFA release. High-plasma FFA concentrations enhance glucose-stimulated insulin secretion (38), which can then feed back to inhibit lipolysis. High FFA also suppresses growth hormone secretion (39), which in turn can reduce lipolysis. There is also evidence that FFAs may themselves directly inhibit adipose tissue FFA release (40). In contrast, lowering plasma FFA concentrations decreases glucose-mediated insulin secretion (41) and can increase plasma growth hormone concentrations (42). Therefore, changes in plasma FFA concentrations influence the secretion of lipolytic and anti lipolytic hormones in a counterregulatory fashion. The data from the present study suggest to us that energy expenditure (requirement) is an indicator of the need for FFA as an oxidative fuel. In general, even when individuals are in energy balance, greater energy requirements require greater plasma FFA uptake and oxidation, thereby lowering plasma FFA concentrations and setting in motion counterregulatory responses that maintain plasma FFA. Likewise, lesser energy requirements and fatty acid oxidation would require lesser FFA uptake and thus reduced lipolysis to maintain “normal” plasma FFA concentrations. This feedback loop provides a mechanism that allows energy demand to regulate the release of FFA, an oxidative fuel, into the circulation. The higher basal rate of lipolysis we observed in women can be explained by a higher rate of nonoxidative FFA clearance in women than in men.

In summary, these results have important implications for understanding endogenous fuel mobilization and how it may affect human health. We found that (a) FFA release rates are highly correlated with REE; (b) women have higher rates of FFA release in relationship to energy requirements than do men; (c) plasma FFA concentrations are not different in women and men, indicating greater nonoxidative FFA clearance in women; and (d) increased basal catecholamine concentrations are associated with greater resting FFA release rates. These findings suggest that new approaches will be required to assess how variations in lipolytic activity might impact on obesity-related disease. The study of adipose tissue lipolytic rates should account for REE and sex if the goal is to identify abnormalities in the regulation of adipose tissue lipolysis. The greater relative FFA availability in women compared with men at comparable FFA concentrations could be a distinct metabolic advantage at times of increasing needs for lipid fuel, such as during exercise, stress, or starvation.

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