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Human aging is associated with altered TNF-α production during hyperglycemia and hyperinsulinemia

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Kirwan, John P., Raj K. Krishnan, James A. Weaver, Luis F. Del Aguila, and William J. Evans. Human aging is associated with altered TNF-α production during hyperglycemia and hyperinsulinemia. *Am J Physiol Endocrinol Metab* 281: E1137–E1143, 2001.—Changes in tumor necrosis factor-α (TNF-α) may provide a mechanism to explain impaired glucose metabolism with advancing age. Hyperglycemic clamps (180 min, 10 mM) were performed on seven older [67 ± 2 yr; body mass index (BMI) 24.7 ± 1.0 kg/m^2] and seven younger (22 ± 1 yr; BMI 21.8 ± 1.3 kg/m^2) healthy sedentary males with normal glucose tolerance. TNF-α production at basal and at the end of 180 min of hyperglycemia and hyperinsulinemia was measured ex vivo from lipopolysaccharide-stimulated (1 ng/ml) peripheral blood mononuclear cells. Plasma glucose, insulin, and C-peptide levels were similar in both groups at basal and during the last 30 min of the hyperglycemic clamp. Glucose infusion rates were lower ($P < 0.004$) in the older group compared with the young, indicating decreased insulin action among the older subjects. Basal TNF-α secretion was similar in older and younger subjects. TNF-α was suppressed ($P < 0.02$) in the younger group (230 ± 46 vs. 126 ± 49 pg/ml; basal vs. clamp) but not in the older group (153 ± 37 vs. 182 ± 42 pg/ml), with significant group differences in response ($P < 0.05$). A significant correlation was observed between the level of suppression in TNF-α production and insulin action (Kendall's rank, $\tau = 0.40$, $P < 0.05$). Furthermore, the TNF-α response during the clamp was related to fat mass ($r = 0.88$, $P < 0.001$) and abdominal fat ($r = 0.81$, $P < 0.003$). In conclusion, these findings suggest a possible mechanism by which TNF-α may modulate glucose metabolism in younger people. Aging and modest increases in adiposity prevent the "normal" suppression of TNF-α production after a sustained postprandial-like hyperglycemic-hyperinsulinemic stimulus, which may contribute in part to the decline in insulin sensitivity in older men.

insulin resistance; obesity; diabetes; abdominal adiposity

HUMAN AGING is associated with the development of glucose intolerance (2), abnormal pancreatic β-cell secretion (3, 18, 25, 32), and insulin resistance (6, 8, 26). However, it is unclear as to the mechanism responsible for these changes as people age. Recent investigations have implicated the cytokine tumor necrosis factor (TNF)-α as a modulator of glucose metabolism. Particularly, TNF-α has been associated with the metabolic defects related to insulin resistance. *In vitro* studies show that TNF-α can induce insulin resistance and downregulate insulin receptor signaling in cultured adipocytes (22), hepatocytes (14), and skeletal muscle (10). Furthermore, increased TNF-α is associated with insulin resistance in obesity (23), sepsis (29), after muscle damage (11), and with age-associated muscle wasting (17). Interestingly, elevated plasma TNF-α levels have been observed in older men and women (37), and increased TNF-α protein expression has been reported in adipose tissue and skeletal muscle of obese and diabetic humans (24, 35, 40). Indeed, TNF-α may provide a link to explain the impaired glucose metabolism that is seen with advancing age. Furthermore, the normal age-related increases in body fat and abdominal adiposity (41) may be related to the effects of age on TNF-α and its role as a glucoregulatory modulator.

The purpose of this investigation was to determine the effects of physiologically elevated levels of glucose and insulin on TNF-α production in healthy sedentary older men. To determine the effects of age on TNF-α production, the responses among these older men were compared with those of a group of healthy sedentary younger men. Because the focus of the investigation was to examine underlying abnormalities in TNF-α and glucoregulation among successfully aging older adults, the older and younger men had normal glucose tolerance and pancreatic β-cell secretion and were not obese.

METHODS

Subjects. Fourteen men (7 younger, age 21–28 yr, and 7 older, age 59–75 yr) participated in this study. All subjects were screened for acute and chronic diseases, and none was taking medications that would affect carbohydrate metabo-

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lism or immune function. In addition, all of the subjects were sedentary, with a similar activity level between groups, as assessed by a physical activity questionnaire. None of the subjects was involved in any regular exercise regimen for at least 6 mo before the time of testing. All participants had a normal plasma glucose response to a 75-g oral glucose tolerance test (1). All of the subjects provided written informed consent in accordance with The Pennsylvania State University guidelines for the protection of human subjects.

Height without shoes was measured to the nearest 1.0 cm. Body weight was measured to the nearest 0.1 kg. Body circumferences were measured at the waist (level of the umbilicus) and hip (point of widest circumference around the buttocks). Waist circumference and waist-to-hip ratio were used to estimate abdominal adiposity (26). Body density and body fat were determined by hydrostatic weighing after an overnight fast as previously described (28).

Study design. All of the trials included residence at the General Clinical Research Center (GCRC) for three nights and two consecutive days (*day 1* and *day 2*). Subjects consumed a balanced diet and received all of their meals from the GCRC for the 2 days before the clamp (young, 3,704 ± 94 kcal; old, 2,613 ± 130 kcal). During these 2 days, the subjects maintained a normal level of activity and did not exercise. Hyperglycemic clamps were performed on *day 3* of residence.

Hyperglycemic clamp. The hyperglycemic clamp (180 min, 10.0 mM) was performed as described by DeFronzo et al. (9). After an overnight fast (~12 h), the subjects voided morning urine and were weighed. An 18-gauge polyethylene catheter was inserted in an antecubital vein for the infusion of glucose (20% dextrose). A second 20-gauge polyethylene catheter was inserted in retrograde fashion in a dorsal hand vein, and the hand was warmed in a heated box (~65°C) for sampling of arterialized venous blood. Baseline blood samples were drawn for glucose, insulin, and C-peptide determination. Subsequently, plasma glucose concentrations were raised to 10.0 mM within 15 min by using a primed glucose infusion with a variable-speed infusion pump (Harvard Apparatus, South Natick, MA). Plasma glucose concentrations were maintained at 10.0 mM for a further 165 min by a variable-rate infusion based on the prevailing plasma glucose concentration. Blood samples (0.5 ml) were drawn every 5 min and were assayed immediately by the glucose oxidase method (Beckman Instruments, Fullerton, CA). The glucose concentrations were used to adjust the infusion rate throughout the clamp procedure. In addition, blood samples (3.0 ml) were drawn every 2 min for the first 10 min (0–10 min) and every 15 min for the remainder of the clamp procedure (15–180 min) to determine insulin and C-peptide concentrations during the first (0–10 min) and second (10–180 min) phases of pancreatic secretion. At the conclusion of the clamp, a urine sample was obtained for the determination of glucose concentration.

Analytical methods. Mononuclear cell (MNC) isolation and culture were performed on 20 ml of arterialized venous blood, which was obtained at 0 and 180 min of the clamp. The cells were isolated by Ficoll-Hypaque centrifugation (4), washed two times in pyrogen-free saline, suspended in RPMI (100 U/ml penicillin, 100 μg/ml streptomycin, and 0.3 mg/ml L-glutamine) with serum substitute TCH, and seeded in coated culture plates (2.5×10^6 cells/ml). The cells were then incubated (humidified, 5% CO_2, 37°C) for 24 h with lipopolysaccharide endotoxin (LPS, 1 ng/ml). After incubation, supernatants (10,000 *g* for 1 min) were obtained and stored at −70°C until analysis. TNF-α concentrations were measured in duplicate by ELISA (Endogen, Woburn, MA). Complete blood counts were obtained using a Coulter Counter (Coulter Instruments). Plasma insulin and C-peptide concentrations were determined in duplicate by double-antibody RIA using commercial kits (Linco Research, St. Charles, MO, and Diagnostic Products, Los Angeles, CA). To reduce interassay variability, all samples for each subject were run in the same assay.

Statistics. The MIXED procedure for the Statistical Analysis System (SAS Institute, Cary, NC) was used for ANOVA by the rank transformation (nonparametric) approach to identify statistical differences in the data. Primary dependent variables were analyzed by two-way repeated-measures ANOVA with the following main effects: group (young and old) and trial (basal and clamp). Descriptive data were analyzed using a one-way ANOVA. Model-adjusted *P* values from a comparison of the least-squared means were used to determine differences between basal and clamp within groups. Group-by-trial interaction was used to demonstrate group differences in measured responses when the basal was compared with the clamp within groups. Spearman product-moment correlations were used to determine the relationship between the TNF-α response and body composition. Kendall's rank, τ, was used to determine the relationship between the change in TNF-α production and estimated insulin action. All values are expressed as means ± SE. An α-level of 0.05 was used to determine statistical significance.

RESULTS

Subjects were similar in weight, fat-free mass, and body mass index (BMI), but the older group had a higher fat mass, waist circumference, and waist-to-hip ratio (Table 1). All subjects had a normal response to an oral glucose tolerance test (2-h value, 5.6 ± 0.4 mM for the older group and 5.7 ± 0.5 mM for the younger group). Basal glucose, insulin, and C-peptide levels were within normal limits and were similar between the two age groups (Table 2). Both groups were clamped at similar glucose levels, and there was no difference in the insulin and C-peptide response to hyperglycemia (Table 2). However, the glucose infusion rate that was required to maintain hyperglycemia in the older group was less than the rate in the younger group (10.0 ± 0.1 vs. 6.7 ± 0.6 mg·kg fat-free mass^{-1}·min^{-1}, $P < 0.004$). To estimate insulin action in the two groups, the glucose disposal rate (M value, calculated from the glucose infusion rate from 150 to 180 min and adjusted for the glucose equivalent space and urinary glucose loss, if any) was divided by the corresponding insulin concentration (I). Insulin action was reduced ($P < 0.02$) in the older men (Fig. 1).

Table 1. *Subject characteristics*

	Younger	Older
Age, yr	22 ± 1	67 ± 2
Height, cm	181.4 ± 1.1	172.7 ± 2.3*
Body weight, kg	71.7 ± 3.8	74.0 ± 4.4
Body mass index, kg/m^2	21.8 ± 1.3	24.7 ± 1.0
Body fat, %	13.9 ± 1.9	22.3 ± 1.8*
Fat mass, kg	9.5 ± 1.5	16.2 ± 1.5*
Fat-free mass, kg	62.1 ± 2.8	57.8 ± 3.3
Waist circumference, cm	75.2 ± 2.6	89.0 ± 2.9*
Waist-to-hip ratio	0.82 ± 0.03	0.91 ± 0.02*

Values are expressed as means ± SE; $n = 7$ subjects in each group. *Significantly different from younger group, $P < 0.05$.

Table 2. *Plasma glucose, insulin, C-peptide, and TNF-α levels and monocyte TNF-α production at rest and during the hyperglycemic clamp in older and younger men*

	Basal	Clamp	Δ
Glucose, mM			
Younger	5.1 ± 0.1	10 ± 0.1	+4.9
Older	5.1 ± 0.1	10 ± 0.0	+4.9
Insulin, pM			
Younger	50 ± 2	185 ± 21	+135
Older	54 ± 4	208 ± 36	+154
C-peptide, nM			
Younger	0.36 ± 0.60	1.35 ± 0.23	+0.99
Older	0.42 ± 0.11	1.34 ± 0.22	+0.92
Plasma TNF-α, pg/ml			
Younger	3.40 ± 0.29	3.17 ± 0.40	−0.23
Older	3.82 ± 0.34	3.80 ± 0.30	−0.02
Monocyte TNF-α, pg/ml			
Younger	230 ± 46	126 ± 49*	−104
Older	153 ± 37	182 ± 42	+29†

Values are expressed as means ± SE. TNF-α, tumor necrosis factor-α; Basal, preclamp; Clamp, after 180 min of hyperglycemia and hyperinsulinemia. Δ, calculated difference between means for clamp − basal. Plasma glucose, insulin, and C-peptide concentrations are calculated means for 150–180 min. *Clamp significantly lower than basal, $P < 0.02$. †Significantly different response than the younger group, $P < 0.05$.

TNF-α production was similar for both groups in the basal state. However, hyperglycemia and hyperinsulinemia resulted in a suppression of TNF-α secretion in the young subjects but no change in the older men (Table 2 and Fig. 2). The response between the two groups was significantly different ($P < 0.05$). Univariate analysis revealed a direct relationship between TNF-α production after hyperglycemia-hyperinsulinemia and both fat mass ($r = 0.88$, $P < 0.001$) and waist circumference ($r = 0.81$, $P < 0.003$) for the combined groups (Figs. 3 and 4). In addition, the amount of suppression in TNF-α production during hyperglycemia-hyperinsulinemia was directly associated with insulin action, estimated from the M-to-I ratio during the clamp (Kendall's rank, $\tau = 0.40$, $P < 0.05$). As shown in

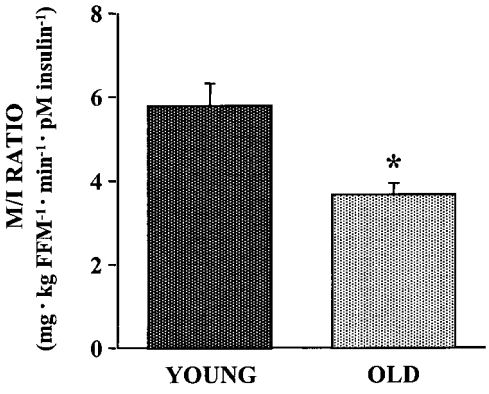

Fig. 1. Estimate of insulin sensitivity based on the ratio of the glucose disposal rate (M) to insulin concentration (I) during the hyperglycemic-hyperinsulinemic clamp. M and I were calculated for the final 150–180 min of the clamp. Units are expressed relative to fat-free mass (FFM). *Significantly lower sensitivity in the older group, $P < 0.02$.

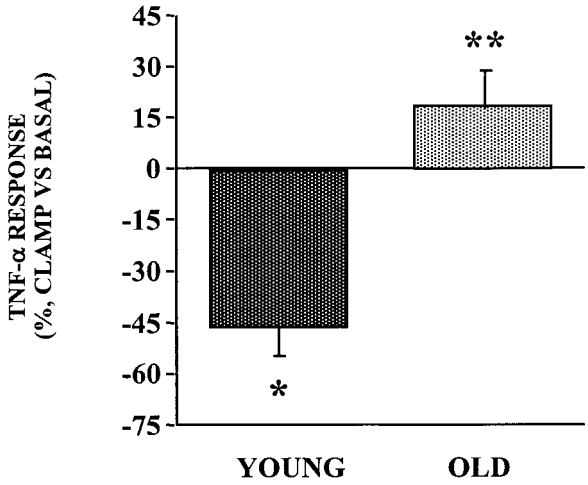

Fig. 2. Tumor necrosis factor (TNF)-α production from monocytes cultured with lipopolysaccharide for 24 h. Basal samples were collected before the clamp, and clamp samples were collected at the end of 180 min of the glucose infusion. *Clamp TNF-α production was significantly lower than basal in the young group, $P < 0.02$. **TNF-α response to hyperglycemia-hyperinsulinemia in the older group was significantly different from that of the young group, $P < 0.05$.

Table 2, plasma TNF-α concentrations followed similar trends as monocyte TNF-α production in both groups; however, the decrease after hyperglycemia-hyperinsulinemia for the young was not significant ($P = 0.20$). Basal plasma TNF-α concentrations were not significantly increased in the older compared with younger men ($P = 0.18$).

Total MNC, lymphocyte, monocyte, and granulocyte numbers were similar for both the older and younger groups under basal conditions (Table 3). The hyperglycemic-hyperinsulinemic conditions that prevailed during the clamp resulted in a significant decrease in total MNC and lymphocyte numbers in both groups. Monocyte number was decreased in the older group but not

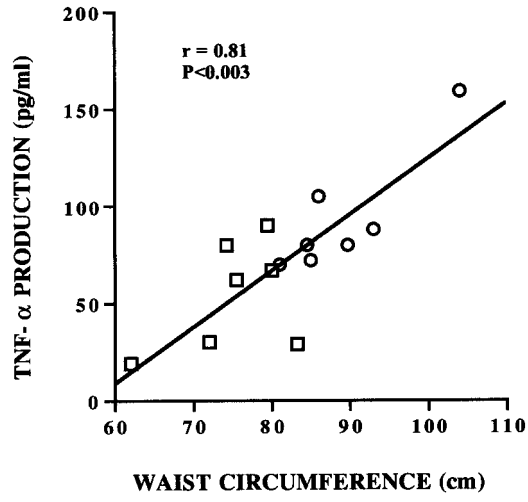

Fig. 3. Correlation between abdominal adiposity and the suppression of TNF-α production during the clamp. Data are shown for 14 men with normal glucose tolerance. ○, Older men; □, younger men. TNF-α was measured from lipopolysaccaride-stimulated monocytes obtained at 0 and 180 min of the clamp. Abdominal adiposity was estimated from waist circumference.

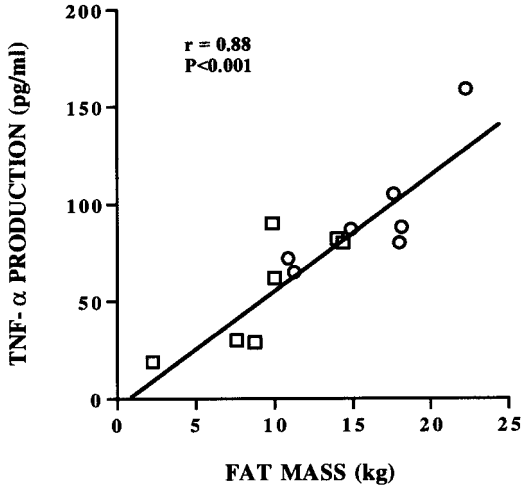

Fig. 4. Correlation between fat mass and the suppression of TNF-α production during the clamp. Data are shown for 14 men with normal glucose tolerance. ○, Older men; □, younger men. TNF-α was measured from lipopolysaccharide-stimulated monocytes obtained at 0 and 180 min of the clamp. Fat mass was estimated from hydrostatic weighing.-

the young. Granulocyte number was not changed in either group.

DISCUSSION

The present study is the first to show that, although older healthy men may have normal glucose tolerance, there is an underlying abnormality in TNF-α production when presented with a sustained physiological hyperglycemic and hyperinsulinemic challenge. Compared with a group of healthy young men, the older men failed to show a "normal" suppression in TNF-α production under postprandial-like conditions. Our data provide further support for the role of TNF-α as a modulator of insulin-mediated glucose metabolism, and, indeed, TNF-α may contribute to the decline in insulin action observed among these older men. Although the older men were relatively lean, fat mass and abdominal fat were significantly greater than in the young group. Furthermore, the independent associations between TNF-α and both fat mass and waist circumference suggest that the increased body fat, particularly abdominal fat, may be a key determinant of the observed differences in TNF-α secretion and its potential role in modulating insulin action.

The effect of in vivo hyperglycemia, or simultaneous hyperglycemia and hyperinsulinemia, on TNF-α secretion in humans remains unknown, despite the fact that these conditions are most reflective of the postprandial state and may have a considerable bearing on the development of insulin resistance. Indeed, much of what we know about the relationship between TNF-α and glucose metabolism is based on studies that have examined how TNF-α affects insulin receptor signaling and consequently glucose uptake or how TNF-α stimulates insulin secretion from β-cells (19, 20, 36). In vitro studies using supraphysiological glucose concentrations (>22 mM) have reported an increase in TNF-α secretion from healthy human peripheral blood MNCs (20, 36). The exact mechanism by which hyperglycemia alters TNF-α secretion in these cells is unknown, but hyperglycemia can stimulate transcription of TNF-α mRNA and protein turnover, with a possible mechanism being the increased osmolarity that is associated with high glucose (36).

In contrast to these in vitro studies with hyperglycemia, healthy sedentary young men in the present study showed an ~45% decrease in TNF-α secretion after hyperglycemia and hyperinsulinemia. We took these data to represent the normal in vivo TNF-α response to 3 h of hyperglycemia coupled with hyperinsulinemia in males. So, is there a physiological benefit to decreased TNF-α secretion after hyperglycemia and hyperinsulinemia in these young men? It is now well documented that TNF-α can impair insulin receptor signaling in adipose tissue and skeletal muscle, thereby decreasing insulin-mediated glucose uptake (10, 11, 14, 22, 23, 29). It was recently shown that TNF-α promotes Ser^{307} phosphorylation of insulin receptor substrate (IRS)-1, which impairs IRS-1 association with the insulin receptor, thus inhibiting downstream insulin signaling (39). Furthermore, TNF-α production by monocytes from healthy young men is positively correlated with the activity of the downstream protein phosphatidylinositol 3-kinase in skeletal muscle during transient insulin resistance (11). Because TNF-α appears to play an important role in reducing insulin-stimulated glucose metabolism, it may be beneficial to be able to control TNF-α production when there is a need to increase glucose disposal. The decrease in TNF-α secretion among the younger group suggests a novel mechanism whereby TNF-α suppression may help to modulate and facilitate glucose disposal.

In contrast, healthy sedentary older men failed to suppress TNF-α secretion during similar hyperglycemic and hyperinsulinemic conditions. Furthermore, even though the older men had normal glucose tolerance and a similar insulin secretion as the younger subjects, glucose infusion rates were lower in the older

Table 3. *Blood cell counts at rest and during the hyperglycemic clamp in older and younger men*

	Basal	Clamp	Δ
MNC ($\times 10^3$ cells/μl)			
Younger	6.0 ± 0.5	5.2 ± 0.7*	−0.8
Older	6.6 ± 0.4	6.0 ± 0.3*	−0.6
Lymphocyte number ($\times 10^3$ cells/μl)			
Younger	1.9 ± 0.2	1.2 ± 0.2*	−0.7
Older	1.8 ± 0.2	1.7 ± 0.2*	−0.1†
Monocyte number ($\times 10^3$ cells/μl)			
Younger	0.3 ± 0.1	0.3 ± 0.0	0.0
Older	0.5 ± 0.1	0.3 ± 0.0*	−0.2
Granulocyte number ($\times 10^3$ cells/μl)			
Younger	3.8 ± 0.5	3.8 ± 0.8	0.0
Older	4.3 ± 0.2	4.1 ± 0.3	−0.2

Values are expressed as means ± SE. MNC, mononuclear cells. *Clamp significantly different from basal, $P \leq 0.05$. †Significantly different response than the younger group, $P < 0.05$.

group. The glucose infusion rates were calculated relative to the prevailing insulin concentration (M-to-I ratio) and were used as a surrogate measure of insulin resistance. As shown previously (13, 25, 28), insulin resistance was increased among the older group. The correlation between the degree of TNF-α suppression and insulin action suggests that the mechanism responsible for impaired glucose metabolism in the older men may be related to TNF-α. It has been shown that plasma TNF-α is increased with advancing age, and the increase is associated with insulin resistance (37). A similar observation has been reported when comparing plasma TNF-α levels in young and old rats (33). Furthermore, when old rats are infused with TNF-α, they experience increased insulin resistance (33). When these observations are coupled with TNF-α production data from the present study, the emerging hypothesis is that the decline in insulin sensitivity with advancing age may be related to impaired suppression of TNF-α secretion, particularly during postprandial periods when glucose and insulin levels are elevated.

The effect of aging per se on TNF-α secretion under basal conditions has not been clearly established (38). In the present study, data collected before the clamp allowed us to compare TNF-α production in older vs. younger men. Although TNF-α secretion was ~33% lower in the older compared with the younger group, the difference was not significant. Mooradian et al. (34) also reported ~40% lower TNF-α secretion when comparing young men with an older group of subjects, which again was not significantly different. Roubenoff et al. (38) found that LPS-stimulated TNF-α secretion was not increased with age. In contrast, several studies report a decrease in LPS-induced TNF-α production with age (5, 12, 16). The similarity in the TNF-α response between the young and old groups under fasting conditions in the present study, and the difference between these results and the findings of other investigators who have shown a decrease in TNF-α secretion with age, may be a function of the relative health status of the study subjects. The older subjects in our study were very healthy, took no medications, and had no acute or chronic cardiovascular or metabolic abnormalities. They were in the healthy category according to NHANES III data, were not overweight, and had a BMI of <25 kg/m^2. Approximately 30% of 65- to 74-yr-old men in the United States have a BMI <25 kg/m^2 (27). We also controlled for the activity level before the procedures and standardized the diet and residence before testing. Thus these data are relevant to healthy aging per se and are not influenced by age-related diseases or antecedent diet and physical activity.

The change in TNF-α secretion after hyperglycemia and hyperinsulinemia was directly related to fat mass and abdominal fat. Although both groups of men were relatively lean, the older men did have greater fat mass and waist circumference compared with the younger group. Thus it appears that, with advancing age, even modest increases in adiposity, especially in the abdominal region, may contribute to the inability to suppress TNF-α secretion during hyperglycemia and hyperinsulinemia. These data extend our previous observations (25, 26) and those of Coon et al. (7) showing that age-related changes in insulin resistance were a function of abdominal adiposity. The present data are particularly interesting because they provide a potential mechanism to explain these observations and are supported by earlier reports linking obesity, elevated TNF-α expression, and insulin resistance (21, 23, 24, 40). The issue of body fat distribution is particularly important in light of a recent report that TNF-α secretion from subcutaneous adipose tissue of obese women is related to insulin-stimulated glucose transport in adipocytes (30). Data from the present study suggest that abdominal adiposity is also associated with altered TNF-α secretion from monocytes in nonobese older men who have experienced an age-related increase in insulin resistance. Additional studies will be required to determine whether being overweight or obese increases or decreases TNF-α further and whether these changes are associated with glucose metabolism.

Fasting MNC numbers were similar in older and younger men and were suppressed to the same extent in both groups after the clamp. Monocytes were reduced in older, but not younger, men after the clamp. The reason for the decrease in the older men is unclear, but it is known that hyperglycemia can induce monocyte adherence to endothelium in diabetic rats through an increase in nonenzymatic glycation adducts (15). It is also known that there is an increased accumulation of advanced glycation end products (AGE) with advancing age, particularly in the presence of diabetes (31, 42). Although we did not measure AGEs in the present study, it is quite likely that there was greater accumulation in the older men, which would in turn facilitate greater adherence of monocytes in response to hyperglycemia and hyperinsulinemia compared with the younger group. Because the binding of AGEs to monocytes initiates cytokine-mediated processes (31), it is also possible that this mechanism may help to explain the different TNF-α response between the young and old groups. It is important to note that, despite differences between pre- and postclamp whole blood monocyte number, the in vitro component of the study allowed us to evaluate TNF-α secretion from equal numbers of monocytes in each group.

In conclusion, we observed an age-related impairment in TNF-α production during hyperglycemia and hyperinsulinemia. The failure to suppress TNF-α secretion among healthy older individuals compared with a younger group represents a unique observation in light of the decline in insulin action with age. Moreover, the association between TNF-α production and both total and abdominal fat suggests that modest increases in adiposity may be responsible for the different TNF-α responses in our groups. Thus the present data add to the growing body of literature drawing relationships between aging, glucose intolerance, changes in body composition, and modulatory factors such as TNF-α. These data raise the intriguing

possibility that, during postprandial periods when glucose and insulin are elevated, TNF-α production is decreased to facilitate glucose uptake by insulin-sensitive tissues. The loss of this postprandial response may be one of the factors contributing to insulin resistance with advancing age.

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