Is obesity an inflammatory disease?

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Background. *Most obese individuals have elevated concentrations of interleukin-6 (IL-6) and tumor necrosis factor-α (TNF-α), markers of inflammation closely associated with diabetes, hypertension, and stroke.*
Hypothesis. *Obesity is a low-grade inflammatory disease, and Roux-en-Y gastric bypass (RYGB) reduces biochemical markers of inflammation and modifies gene expression in hypothalamic food intake/energy-related nuclei and subcutaneous abdominal fat (SAF).*
Methods. *Obesity was induced in 24 3-week-old Sprague Dawley pups fed a high-energy diet (HED). Three groups (n = 8/group) were studied: RYGB, sham-operated pair-fed, and sham-operated ad libitum HED. Controls were nonobese rats fed chow (n = 6). Rats were killed 10 days after operation, and blood was collected to measure corticosterone and SAF and mesenteric fat to measure IL-6, TNF-α, and corticosterone. Total mRNA from arcuate nucleus and SAF purified for gene expression profiling. Data were analyzed with analysis of variance, Mann-Whitney test, and* t *test.*
Results. *Before operation, the body weight of the obese groups was 493 ± 7 g and control = 394 ± 12g. The 10-day postoperative weight was RYGB = 417 ± 21 g, pair-fed = 436 ± 14 g, and ad libitum HED = 484 ± 15 g. Mesenteric and SAF weight decreased in RYGB. Mesenteric/SAF ratio of IL-6, TNF-α, corticosterone, and gene profiling showed decrease of inflammation after RYGB.*
Conclusion. *Gastric bypass reduces biochemical markers of inflammation, suggesting that obesity is an inflammatory condition. (Surgery 2003;134:329-35.)*

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OBESITY IS DUE TO A COMPLEX INTERACTION between genetics, diet, metabolism, and physical activity.[1] Currently, surgical intervention remains the most effective approach in the morbidly obese to achieve a significant and sustained weight loss and a reduction in comorbidities. Obesity is associated with a low-grade systemic inflammation. Adipose tissue synthesizes and secretes cytokines tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6),[2,3] and leptin,[4] whose degree of elevation correlate directly to the degree of obesity.[2-5] Increased expression of TNF-α messenger RNA (mRNA) in subcutaneous abdominal fat (SAF) occurs in obese rodents and human beings.[5,6] Similarly, the expression of IL-6 is elevated in human adipose tissue.[2] Adipose tissue also produces corticosterone, a steroid with potent anti-inflammatory properties. It is believed that abdominal obesity, a marker of the existence of metabolic syndrome X, (obesity, insulin resistance, type II diabetes, hypertension, and hyperlipidemia) is a localized form of Cushing's syndrome. It is possible that the increase in the local production of corticosterone by abdominal adipose tissue is in response to elevated TNF-α and IL-6 production. TNF-α, IL-1, and IL-6 enhance the activity of 11-β hydroxysteroid dehydrogenase type I (11-β HSD-I),[7] which leads to the formation of corticosterone. Thus there appears to be a balance between proinflammatory cytokines (TNF-α, IL-1, and IL-6) and anti-inflammatory corticosterone, a product of 11-β HSD-I activity. Elevated proinflammatory cytokines increased local corticosterone production that leads to increased deposition of fat in the abdomen. Ultimately, this process determines the presence or absence of abdominal obesity, and so the occurrence of metabolic syndrome X.

We therefore hypothesize that obesity is an inflammatory disease, and RYGB reduces body weight with a reduction of biochemical markers of inflammation, together with modification of gene

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expression in hypothalamic food intake/energy related nuclei and SAF.

METHODS

The study was approved by the Committee for the Humane Use of Animals at the State University of New York Upstate Medical University and was in accordance with guidelines established by the National Institutes of Health.

Preoperative procedures. Three-week-old, male Sprague Dawley rats (Taconic Farms, Germantown, NY), weighing 54.9 ± 1.8 g, were acclimated to the constant study environmental conditions: 12/12-hour light/dark cycle (lights on at 06:00), 26° ± 1° C room temperature, and 45% humidity. After acclimation, the 30 rats were initially divided into the control group (n = 6), and the diet-induced obesity (DIO) group (n = 24). Control rats were fed a standard chow diet (diet 5008; Ralston Purina, St Louis, Mo), whereas rats in the DIO group were fed a high-energy (HE) diet (D12266; Research Diets, New Brunswick, NJ) together with a highly palatable liquid diet (Boost, Mead Johnson, Evansville, Ind). The high-energy diet consisted of 4.5 kcal/g, and Boost contained 1.5 kcal/mL. The rats were allowed diets ad libitum and municipal tap water. To avoid regionally specific effects of sex-steroid hormones on adiposity metabolism, we used male rats. One week before the operation, rats were housed in individual metabolic cages. On the basis of the amount of body weight that was gained, the DIO rats were divided into 3 groups: RYGB (n = 8); sham-operated, who continued on as-desired high-energy diet (HE ad lib; n = 8); and sham-operated pair-fed (PF; n = 8). The 24 rats were stratified according to weight and randomly assigned within each strata to each of the 3 groups. This stratified randomization technique ensures that the mean weight of each group is approximately identical. (The initial weights of each group [mean ± SE] were as follows: RYGB = 496.9 ± 16.3 g; HE ad lib = 492.1 ± 13.8 g; PF = 499.8 ± 14.9 g; n= 8 per group; $P > .05$ via paired t test.) Before operation the rats were deprived of feed for 12 hours. Rats were anesthetized with ketamine and xylazine mixture (150:30 mg/mL) via intramuscular injection (0.7 mL/kg) to obtain effective anesthesia.

Operations

RYGB. As shown in Figure 1 and as previously described in detail,[8] 2 double rows of titanium staple lines (TRH30-4.8, Ethicon, Cincinnati, Ohio) were placed between the lesser and greater curvature of the stomach, creating a 20% gastric pouch. The gastric bypass staple line was reinforced with multiple interrupted 4-0 polyglactin sutures (Ethicon) between the double rows of staple line, handsewn, to provide a secure gastric partition. The jejunum was divided at a distance of 16 cm below the ligament of Treitz and a 4- to 5-mm end-to-side gastrojejunostomy was handsewn with interrupted 5-0 polyglactin sutures. The stump of proximal jejunum was closed with running suture, and a 7- to 8-mm side-to-side jejunostomy was also handsewn at a distance of 10 cm below gastrojejunostomy. (The concept of the procedure is patent pending #R1410110.) After irrigation of the abdominal cavity with warm normal saline solution, the wound was closed in layers. After operation, normal saline solution was injected subcutaneously, 20 to 30 mL/day, for 3 days or as necessary to prevent dehydration.

Sham operation. A sham operation was performed on the other 16 obese rats. After celiotomy, the liver, the stomach, and the esophagus were gently manipulated. The jejunum 15 to 20 cm was laid out for the same duration required for the gastric bypass procedure, and the wound was closed in layers.

Postoperative procedures. Rats were allowed to drink and eat 24 hours after operation, but only liquid diet was provided for the first 4 days, followed by coarsely ground high-energy solid diet. Food was provided as desired to the RYGB and HE ad lib groups, but the PF group was given the mean of previous day caloric intake consumed by the RYGB group. Body weight was measured daily, and 10 days after the operation the rats were killed to obtain biochemical data and to measure body fat content. Adipose tissue samples were collected from the identical areas of the rats of all the groups to avoid sampling errors and to ensure uniformity of excision.

Biochemical analysis. Under isoflurane (Forane; Baxter, Chicago, Ill) anesthesia, rats were decapitated, and mixed venous and arterial blood was collected into ethylenediamine tetra-acetic acid Crinsed tubes. The brains were dissected out, and the blood was spun in a centrifuge at 3000 rpm for 10 minutes at 4° C to obtain plasma. The plasma samples were briefly stored at –80° C. Serum concentration of corticosterone was determined by enzyme-immunoassay kit (ALPCO, Windham, NH). The subcutaneous and mesenteric adipose tissues (5 g per sample) were homogenized with a Tissumizer (Tekmar Company, Cincinnati, Ohio) and spun in a centrifuge at 10,000 rpm for 15 minutes at 4° C. The supernatant of the fat tissue was used to measure the following: corticosterone, IL-6, and TNF-α (ALPCO, Windham, NH) with enzyme-immunoassay kits.

Microarray analysis. Qualitative and quantitative changes in gene expression profile analysis of arcu-

ate nucleus (ARC) and subcutaneous abdominal fat were obtained. The analysis of gene expression patterns in these tissues was carried out by use of the GeneChip Rat UG34A Gene Chip (Affymetrix, Santa Clara, Calif) that interrogated more than 8000 genes and expressed sequence tags. To prepare adipose tissues for analysis, a high-speed tissue homogenizer was used to pulverize (liquefy) fat samples. To obtain precise dissections of hypothalamic tissue, brains were removed after decapitation and rapidly frozen by immersion in isopentane at –80° C. Tissue was then cut at 14 μm with a cryostat, mounted onto PEN foil slides (Leica LS AMD, Leipzig, Germany), and stained with a modified rapid Nissel staining procedure. This stain enabled us to determine the precise location of the arcuate nuclei and then automatically cut these out by use of laser micro dissector software (Leica LS AMD). RNA was extracted and purified from either subcutaneous abdominal fat or ARC with a 2-step protocol that combines the Trizol method with the RNeasy kit (Qiagen Inc, Valencia Calif). The quality and quantity of the purified RNA was assessed with the RNA Lab Chip Kit with the Agilent Technologies Bioanalyzer system (Agilent Technologies, Palo Alto, Calif).

In the labeling reactions, total RNA was reverse transcribed, and second strand cDNA was synthesized according to the Ambion MegaScript protocol (Ambion, Austin, Tex) with a T7-$(dT)_{24}$ primer. The double-stranded cDNA reaction was purified with a Qiagen column and used for in vitro transcription synthesis of biotin-label cRNA. In the analysis of gene expression in ARC, 2 rounds of aRNA amplification were performed on each sample. After purification of biotinylated cRNA, 15 μg of this product was fragmented randomly to 35 to 200 bases (94° C, 35 minutes in fragmentation buffer), added to a hybridization cocktail that also contained known concentration of spiked in controls, heated (95° C, 5 minutes), equilibrated (45° C, 5 minutes), and spun in a centrifuge at maximum speed at room temperature for 5 minutes.

Samples were then injected into the Rat U34 GeneChip array (Affymetrix, Santa Clara, Calif) for incubation at 45° C for 16 to 19 hours with rotation of 60 rpm in a hybridization oven 640 (Affymetrix, Santa Clara, Calif). Probe arrays were washed and stained on the Affymetrix Fluidics Station 400 following the EukGE-WS2 protocol. Fluorescent images were scanned and acquired with the Hewlett-Packard G2500A Gene Array Scanner and GeneChip software (Affymetrix). Data obtained by microarray were analyzed with GeneSpring (Silicon Genetics, Redwood City, Calif) software.

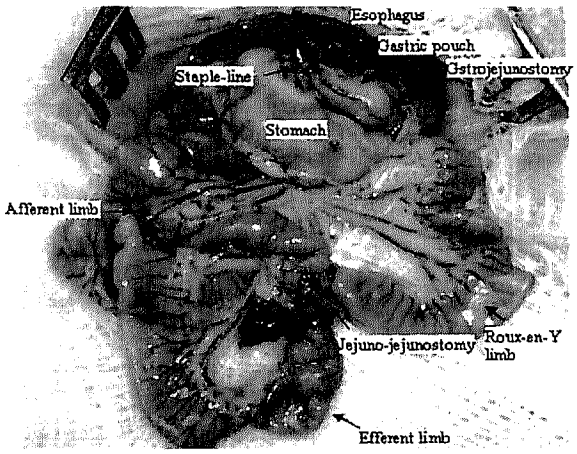

Fig 1. RYGB in rat model. (Patent pending #R1410110)

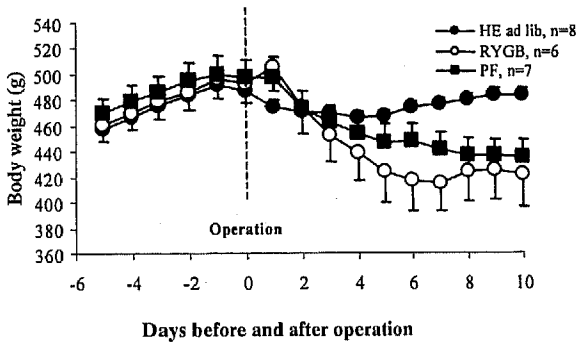

Fig 2. Body weight changes after operation in rats with DIO.

Data were normalized with the chow diet group used as control.

Statistical analysis. The relationship of changes in body weight was analyzed with analysis of variance, and the biochemical data were analyzed post hoc by Mann-Whitney and Student's *t* test. Microarray analysis data were expressed as magnitude changes relative to control group. Data are expressed as mean ± SE.

RESULTS

Operative complications. Two rats from the RYGB group died of anastomotic leakage at days 3 and 4 after operation. One rat from the PF group died during anesthesia.

Body weight changes. Twenty-four Sprague-Dawley pups (3 to 4 weeks) were fed the high-energy diet for 7 weeks to induce obesity (DIO), and 6 were fed chow serving as control. Body weight at 11 weeks in the DIO group was 493 ± 7 g and in the control group was 394 ± 12 g. As shown in Figure 2, the body weight of rats decreased during the 2 to 4

Table I. Fat tissue measurements at euthanasia

	Subcutaneous (g)	*Retroperitoneal (g)*	*Mesentery (g)*	*Epididymal (g)*	*Liver (mg/g)*
Control	5.8 ± 0.4*	3.9 ± 0.5*	2.5 ± 0.2*	3.5 ± 0.3*	—
He ad lib	13.1 ± 1.2	11.5 ± 1	7.8 ± 0.8	8.6 ± 0.8	55.6 ± 3.8
PF	11.2 ± 1.6	8.7 ± 1.1	6.9 ± 1	7.7 ± 0.9	36.4 ± 7.6
RYGB	7.3 ± 0.4†	8.1 ± 1.2†	5.8 ± 1.1	8.7 ± 1.2	42.4 ± 5.4

Value is shown as mean ± SE. $P < .05$.
*Control vs HE ad lib, PF, and RYGB.
†RYGB vs HE ad lib

Table II. Serum and fat tissue corticosterone concentration

Corticosterone	*RYGB (n=6)*	*HE ad lib (n=8)*	*PF (n=7)*	*Control (n=6)*
Serum (ng/mL)	38.1 ± 6.7*	199.6 ± 59.2	339.6 ± 83.5	256 ± 78.1
SAF (μg/g)	2.8 ± 2	1.0 ± 0.2	1.6 ± 0.3	1.2 ± 0.3
Mesentery (μg/g)	8.4 ± 4	1.4 ± 0.3	4.9 ± 2.6	6.2 ± 2.4
Mes/SAF	3.0†	1.4‡	3.1	5.2

Value is shown as mean ± SE. $P < .05$.
*RYGB vs HE ad lib, PF and control.
†RYGB vs HE ad lib.
‡HE ad lib vs control

postoperative days as a result of anesthesia and the stress effects of operation. Consequently, the body weight 10 days after operation was 417 ± 21 g in the RYGB, 436 ± 14 g in the PF, and 484 ± 15 g in the HE ad lib groups. The weight loss in the RYGB and PF groups compared with the HE ad lib group was significant ($P < .05$). The control group was not subjected to any surgical procedure, serving as control. There was no difference in weight loss in the RYGB versus PF groups ($P > .05$).

Adipose tissue and lipid content. In general, accumulation or deposition of adipose tissue is not uniform in various compartments especially when human subjects and animals become obese. For instance, as subjects become obese, accumulation of mesenteric/abdominal fat is relatively more compared with subcutaneous fat. The exact reason for this is not clear. It is possible that differential accumulation/deposition of fat could be due to local factors. Some of these include local production of corticosterone (caused by 11-β HSD type I activity), lipoprotein lipase, and cytokines, which can influence fatty acid/adipose tissue metabolism. It is known that abdominal adipose tissue has high 11-βHSD type I activity compared with subcutaneous adipose tissue. In view of this, we measured the body fat accumulation in different areas in this study rather than to simply measure the total body weight.

As shown in Table I, subcutaneous abdominal, retroperitoneal, mesenteric and epididymal and hepatic fat were measured. As expected, the fat content in DIO rats (HE ad lib, PF, and RYGB groups) was higher than in control rats on the chow diet, ($P < .05$). The RYGB operation led to a significant loss of subcutaneous and retroperitoneal fat ($P < .05$) when compared with sham-operated obese rats (HE ad lib group). The mesenteric and liver fat contents were decreased, but not significantly. Conversely, no differences were found in the measurement of epididymal fat after RYGB.

Biochemical indexes. Serum corticosterone was decreased after RYGB when compared with the HE ad lib, PF, and control groups and was increased in PF versus RYGB ($P < .05$). Corticosterone concentration in subcutaneous and mesenteric fat did not show statistical differences among groups (Table II), although the corticosterone concentrations in the RYGB group were much closer to those seen in the controls compared with the HE ad lib and PF groups.

The concentrations of IL-6 and TNF-α after the surgical procedures in the RYGB, HE ad lib, and PF groups were higher than in control rats not subjected to any previous surgical intervention. Concentration of IL-6 was higher in SAF and mesenteric fat in RYGB when compared with control ($P < .05$), and not significant when compared with the HE ad lib and PF groups (Table III). There was no significant difference of TNF-α concentration between the RYGB and HE ad lib, PF and control groups. The ratio mesenteric/SAF concentra-

Table III. Biochemical indexes of inflammation in adipose tissues.

Indexes	*Adipose tissue*	*RYGB (n=6)*	*HE ad lib (n=8)*	*PF (n=7)*	*Control (n=6)*
IL-6 (ng/g)	SAF	7.5 ± 2.3*	2.0 ± 0.5	3 ± 1.2	1.6 ± 0.7
	Mesentery	15.6 ± 4.1*	6.9 ± 3	7.2 ± 2.4	3.2 ± 0.5
TNF-α (ng/g)	SAF	169.2 ± 42.2†	105.7 ± 18.2	79.4 ± 16	103.2 ± 29
	Mesentery	31.7 ± 6.2	35.6 ± 5.3	47.5 ± 4.3	30.9 ± 19.1

Value is shown as mean ±SE. $P < .05$.

*RYGB vs control.

†$P < .05$ compared with PF.

tion of IL-6 and TNF-α in RYGB was smaller than in obese rats subjected to laparotomy (HE ad lib and PF groups), and these ratios are much closer to those seen in control rats. However, TNF-α in RYGB versus PF was significantly increased ($P < .05$), as shown in Table III.

Corticosterone is an anti-inflammatory, whereas TNF-α and IL-6 are proinflammatory molecules. Hence, the final balance between the proinflammatory and anti-inflammatory events in any given local tissue depends on the balance between TNF-α versus corticosterone, and between IL-6 versus corticosterone. A ratio between TNF-α and corticosterone and between IL-6 and corticosterone that is less than the control value indicates that the balance of events is tilted more toward suppression of inflammation. If the ratio is more than the control, it indicates that inflammation is actively present and ongoing. The data in Table IV show that TNF-α/corticosterone ratios in subcutaneous and abdominal fat are reduced in the PF and in the RYGB groups when compared with the HE ad lib group. The IL-6/corticosterone ratio in mesenteric adipose tissue was decreased to 1.8 in RYGB when compared with the HE ad lib group (4.9), and more comparable with the normal ratio of 0.5 seen in the control group. Although, in subcutaneous abdominal fat, the IL-6/corticosterone ratio was higher in RYGB than in the HE ad lib and control groups (Table IV).

Microarray analysis. To compare our microarray data, the gene expression in the arcuate nucleus was normalized relative to rats on chow diet. Data are expressed as magnitude changes relative to the control group in Table V. The IL-6 expression in ARC was decreased, and SAF was increased in all groups when compared with the control group. Comparing the magnitude changes of IL-6 in ARC in the RYGB (–1.09), HE ad lib (–1.89), and PF (–2.30) groups, the variation in RYGB is closer to normal. In SAF, the IL-6 expression in the HE ad lib group was 13-fold higher than in the control group, and after RYGB, decreased to 8.5. Comparing the magnitude of TNF-α expression change in ARC and SAF, the expression after RYGB was higher when compared with the other groups (Table V).

Table IV. Biochemical indexes of inflammation in 2 adipose tissues

	SAF		*Mesenteric fat*	
Groups	*IL-6/Cort*	*TNF/Cort*	*IL-6/Cort*	*TNF/Cort*
Control	1.3	86.0	0.5	5.0
RYGB	2.6	60.4	1.8	3.7
HE ad lib	2.0	105.7	4.9	25.4
PF	1.8	49.6	1.4	9.7

DISCUSSION

A reproducible DIO model, developed in our laboratory,[8] was used to study the effect of bariatric operation on well-known inflammatory markers. Our data indicate that after RYGB, changes occur in IL-6, TNF-α and corticosterone that indicate a decrease in inflammatory response. The same changes were confirmed by evaluation of mRNA expression of these substances in subcutaneous abdominal fat and arcuate nucleus, with the microarray technique. Corticosterone is an anti-inflammatory molecule, and after RYGB a decrease occurred when compared with obese rats (the HE ad lib group).

In our study, the concentrations of IL-6 and TNF-α after the surgical procedures in all obese rats, that is, RYGB, HE ad lib and PF groups, were overall higher than in control rats. This can be explained on the basis that the measurements were performed only 10 days after operation, and it is likely that the cytokines were elevated in response to operative trauma. The IL-6 and TNF-α concentrations in subcutaneous and mesenteric fat did not show statistical differences between RYGB group and the obese rats in which a sham operation was performed (the HE ad lib group).

In DIO, the fat tissue accumulates in various body regions, with more mesenteric than subcuta-

Table V. Corrected magnitude changes of IL-6 and TNF-α relative to control.

	IL-6 (ARC)	*TNF-α (ARC)*	*IL-6 (SAF)*	*TNF-α (SAF)*
Control	1	1	1	1
RYGB	−1.09	1.23	8.55	−1.07
HE ad lib	−1.89	−3.44	13.00	−2.24

Table VI. Semiquantitative representation of the degree of inflammation relative to control

	IL-6 (Mes/SAF)	*TNF-α Mes/SAF*	*Inflammation*
Control	2.0	0.3	Control
HE ad lib (obese)	3.5	0.3	↑↑↑↑
PF	2.4	0.6	↑↑↑↑
RYGB	2.1	0.2*	↑↑

Mes, Mesenteric fat.
↑ indicates degree of inflammation.
*RYGB vs PF. $P < .05$.

neous fat deposition. In our study, the subcutaneous fat deposition was 225% higher and the mesenteric deposition was 312% higher in obese high-energy diet control rats (HE al lib) than in nonobese rats (control). As a result of the feedback control between subcutaneous abdominal and mesenteric fat, the concentration of IL-6 was relatively higher in the mesenteric than subcutaneous abdominal fat, reflecting higher inflammation in mesenteric fat. Hence, the use of a ratio of mesenteric to subcutaneous fat will give a more precise physiological picture of the relative inflammation between these sites. The ratios of mesenteric to subcutaneous IL-6 and TNF-α in the RYGB group were smaller than in obese rats fed ad libitium or pair-fed and were more comparable to those seen in the control rats (Table VI). This emphasizes the decrease of inflammation after RYGB.

A dramatic decrease in the plasma concentrations of corticosterone in the RYGB group was noted in comparison to all other groups, especially in reference to the PF group. The decrease in the concentrations of corticosterone after RYGB in comparison to the PF group is particularly interesting because both these groups consumed the same amount of food. The RYGB rats had a small gastric pouch and so were forced to reduce their food intake compared with what they consumed before surgery. Therefore rats in the RYGB group were no longer hungry after consuming less food and are expected to be satiated. In contrast, PF rats have a normal stomach size but were given the same amount of food that the RYGB rats were consuming, which was less, compared to the size of their stomach. Thus PF rats are constantly hungry even after being fed, producing stress and explaining the high concentrations of corticosterone in their plasma. The hunger-related stress stimulates the hypothalamic-pituitary-adrenal axis, as reflected by elevated serum corticosterone concentrations in the PF rats, whereas corresponding changes in tissues take a longer time; hence, the subcutaneous and mesenteric fat corticosterone concentrations did not differ between the RYGB and PF groups (Table II).

Under normal conditions, it is expected that there is a balance that is maintained between proinflammatory and anti-inflammatory molecules in various tissues. Thus it is anticipated that whenever the concentrations of proinflammatory cytokines (TNF-α, IL-6, and IL-1) are elevated, a reciprocal change would occur in the concentrations of corticosterone. Adipose tissue produces TNF-α, IL-6, and IL-1 as well as corticosterone. TNF-α, IL-6, and IL-1 enhance the activity of 11-β HSD-I, an enzyme that is essential for corticosterone formation. On the other hand, corticosterone suppresses the production of TNF-α, IL-6, and IL-1, and so corticosterone suppresses inflammation.[9-11] Thus the balance between corticosterone versus TNF-α, IL-6, and IL-1 determines the degree of inflammation in adipose tissue and for that matter, in any tissue(s).

Functionally, subcutaneous adipose tissue is different from the mesenteric adipose tissue because 11-β HSD-I activity is higher in the mesenteric compared with subcutaneous adipose tissue. It is expected that the degree of inflammation in the subcutaneous and mesenteric adipose tissues will be different even in the same obese animals and human beings. This is supported by the observation that the concentrations of corticosterone, TNF-α, and IL-6 produced by the subcutaneous and mesenteric adipose tissues in the control are different (see Tables II and III). For instance, subcutaneous adipose tissue produced 5 and 2 times less corticosterone and IL-6, respectively, and secreted TNF-α 3-fold higher compared with the mesenteric. Hence, the degree and relative amount of inflammation in different sites of adipose tissue will be known only by comparing the ratios between TNF-α versus corticosterone and IL-6 versus corticosterone in a given tissue, and between different adipose tissue depots (such as subcutaneous vs mesenteric) and comparing the ratio with controls.

Thus we calculated the biochemical indexes of inflammation in adipose tissue in different groups of rats and these results are given in Tables IV and VI. The corticosterone ratio mesenteric/SAF in the RYGB (3.0) and PF (3.1) groups was closer to control (5.2) than the HE ad lib group (1.4) and was significantly higher in RYGB versus HE ad lib ($P < .05$), as shown in Table II.

Because IL-6 and TNF-α have a regulatory control on corticosterone production in the adipose tissue, we also calculated the ratio between IL-6 and corticosterone and between TNF-α and corticosterone concentrations in the subcutaneous and mesenteric adipose tissues. The data shown in Table IV suggest that TNF-α to corticosterone ratio are reduced in the PF and the RYGB groups compared with that in the HE ad lib group in subcutaneous and mesenteric adipose tissues. These results indicate that the balance between corticosterone and TNF-α are restored to near normalcy in the PF and RYGB groups compared with the HE ad lib group, indicating reduction in the inflammation.

TNF-α expression in subcutaneous abdominal fat (–1.07) and arcuate nucleus (1.23) in rats in the RYGB group was higher than in rats in the HE ad lib group (–2.24 and –3.44, respectively), suggesting an increase of the amount of mRNA TNF-α produced. However, some authors have published data suggesting that human subcutaneous adipose tissue does not release a significant amount of TNF-α in vivo.[2] The importance of TNF-α lies in the fact that it induces apoptosis of neurons in the brain. On the basis of this, it is suggested that increases in TNF-α concentrations in the hypothalamic areas of the brain may induce death of neurons concerned with appetite and satiety control, which in turn may lead to loss of control of these factors that might result in obesity. On the other hand, after diet restriction and RYGB, a decrease in TNF-α concentrations occurs and so further neuronal death is unlikely. Because the brain continues to regenerate new neurons from the existing neuronal stem cells, it is anticipated that decreased TNF-α concentrations will result in re-equilibration of the actions of the appetite and satiety centers in the hypothalamus to near normal concentration after weight loss.

In conclusion, our study demonstrates that inflammatory markers, such as IL-6 and TNF-α in fat, and serum corticosterone concentrations are related in obesity. RYGB reduces the biochemical markers of inflammation, which modifies gene expression in ARC and SAF, supporting the concept that obesity is an inflammatory condition.

REFERENCES

1. Das UN, Meguid MM. Nutrition, physical activity and obesity. Lancet 2002;360:1249-50.
2. Mohamed-Ali V, Goodrick S, Rawesh A, Katz DR, Miles JM, Yudkin JS, et al. Subcutaneous adipose tissue releases interleukin-6, but not tumor necrosis factor-α in vivo. J Clin Endocrinol Metab 1997;82:4196-200.
3. Bastard J-P, Jardel C, Bruckert E, Blondy P, Capeau J, Laville M, et al. Elevated levels of interleukins 6 are reduced in serum and subcutaneous adipose tissue of obese women after weight loss. J Clin and Endocrinol Metab 2000; 85:3338-3342.
4. Caro JF, Sinha MK, Kolaczybski JW, Zhang PL, Consdine RV. Leptin: the tale of an obesity gene. Diabetes 1996;45:1455-62.
5. Hotamisligil GS, Shargill NS, Spiegelman BM. Adipose expression of tumor necrosis factor-α: direct role in obesity-linked insulin resistance. Science 1993;259:87-91.
6. Kern PA, Saghizadeh M, Ong JM, Bosch RJ, Deem R, Simsolo RB. The expression of tumor necrosis factor in human adipose tissue. Regulation by obesity, weight loss, and relationship to lipoprotein lipase. J Clin Invest 1995;95:2111-9.
7. Tomlinson JW, Moore J, Cooper MS, Bujalska I, Shahmanesh M, Burt C, et al. Regulation of expression of 11beta-hydroxysteroid dehydrogenase type 1 in adipose tissue: tissue-specific induction by cytokines. Endocrinology 2001;142:1982-9.
8. Xu Y, Ohinata K, Meguid M, Marx W, Tada T, Chen C, et al. Gastric bypass model in the obese rat to study metabolic mechanisms of weight loss. J Surg Res 2002;107:56-63.
9. Das UN. Interaction(s) between essential fatty acids, eicosanoids, cytokines, growth factors, and free radicals: relevance to new therapeutic strategies in rheumatoid arthritis and other collagen vascular diseases. Prostaglandins Leukot Essent Fatty Acids 1991;44:201-10.
10. Han J, Thompson P, Beutler B. Dexamethasone and pentoxifylline inhibit endotoxin-induced cachectin/tumor necrosis factor synthesis at separate points in the signaling pathway. J Exp Med 1990;172:391.
11. Jong EC, Viera PL, Kalinski P, Kapsenberg ML. Corticosteroids inhibit the production of inflammatory mediators in immature monocyte-derived DC and induce the development of tolerogenic DC3. J Leukocyte Biol 1999;66:201.