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Subcutaneous and Visceral Adipose Tissue: Their Relation to the Metabolic Syndrome

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ABSTRACT

Methods for assessment, *e.g.*, anthropometric indicators and imaging techniques, of several phenotypes of human obesity, with special reference to abdominal fat content, have been evaluated. The correlation of fat distribution with age, gender, total body fat, energy balance, adipose tissue lipoprotein lipase and lipolytic activity, adipose tissue receptors, and genetic characteristics are discussed. Several secreted or expressed factors in the adipocyte are evaluated in the context of fat tissue localization. The body fat distribution and the metabolic profile in nonobese and obese individuals is discussed relative to lipolysis, antilypolysis and lipogenesis, insulin sensitivity, and glucose, lipid, and protein metabolism. Finally, the endocrine regulation of abdominal visceral fat in comparison with the adipose tissue localized in other areas is presented. (*Endocrine Reviews* **21:** 697–738, 2000)

I. Introduction

EPIDEMIOLOGICAL studies often report an association between severe obesity and mortality due to increased rates of cardiovascular and cerebrovascular diseases and diabetes (1–4). In moderate obesity, regional distribution appears to be an important indicator for metabolic and cardiovascular alterations since an inconstant correlation between body mass index (BMI) and these disturbances has been found (2, 5). Over the last two decades, studies have reemphasized the notion put forward in 1947 by Vague (6) that obesity is not a homogeneous condition and that the regional distribution of adipose tissue is important to understanding the relation of obesity to disturbances in glucose and lipid metabolism (7). Many prospective studies have shown that excess fat in the upper part of the body (*i.e.*, central or abdominal), considered by Vague (6) as "android or male-type obesity," more often correlates with increased mortality and risk for disorders such as diabetes, hyperlipidemia, hypertension, and atherosclerosis of coronary, cerebral, and peripheral vessels more often than the "gynoid" (lower body or gluteo-femoral or peripheral depot) female-type of fat distribution (8–13). However, in these studies, the body fat distribution was assessed using anthropometric measurements such as skinfolds and waist-to-hip circumference ratios (WHR), particularly the latter. Although the WHR is simple and convenient for epidemiological studies and provides a useful estimation of the proportion of abdominal or upper-body fat (14–16), it does not distinguish between accumulations of deep abdominal (visceral) fat and subcutaneous abdominal fat. Imaging techniques, particularly computed tomography (CT), which clearly distin-

guishes fat from other tissues, allows the measurement of visceral and subcutaneous abdominal fat. Several studies have shown that the detrimental influence of abdominal obesity on metabolic processes is mediated by the intra-abdominal fat depot. For example, the visceral fat area correlated with glucose intolerance in the presence of hyperinsulinemia during an oral glucose tolerance test, suggesting an insulin-resistant state (17–19). In addition, correlation analyses have shown that the effect of accumulation of deep abdominal fat on glucose tolerance was independent from total adiposity and subcutaneous abdominal adipose tissue and that no association was observed between total adiposity and glucose tolerance after control for visceral fat area (18, 19). In their study of a wide range of total body fat in both healthy young (20) and middle-aged (21) men, Park, Märin, and colleagues found that the intraabdominal fat area evaluated by CT was associated with a decrease in insulin sensitivity measured by an euglycemic hyperinsulinemic glucose clamp. In addition to being associated with disturbances in insulin-glucose homeostasis, abdominal obesity has been related to alterations in plasma lipoprotein-lipid levels (22–24), particularly increased plasma triglyceride and low high-density lipoprotein (HDL) cholesterol concentrations, as expected from the association of insulin resistance with disturbances in plasma lipid transport and lipoprotein levels (25, 26).

Although the cause-and-effect association has not been definitively established, the available evidence indicates that visceral fat is an important link between the many facets of the metabolic syndrome: glucose intolerance, hypertension, dyslipidemia, and insulin resistance (27). However, because of the considerable metabolic heterogeneity still remaining among obese patients with similar levels of visceral adipose tissue, it was proposed that genetic susceptibility plays a major role in modulating the risk associated with a given excess of visceral adipose tissue (28). In this regard, visceral obesity should be considered a factor that exacerbates an individual genetic susceptibility to the components of the metabolic syndrome (27). While there is a consensus that visceral fat has a strong association with cardiovascular risk factors, particularly dyslipidemia and hyperinsulinemia (29), the primary importance of visceral adipose tissue vis-à-vis subcutaneous abdominal obesity with regard to insulin sensitivity of glucose metabolism has been challenged by Abate *et al.* (30) and Goodpaster *et al.* (31). These researchers found that abdominal subcutaneous fat, as determined by magnetic resonance imaging and CT, was at least as strong a correlate of insulin sensitivity (evaluated by the euglycemic clamp) as visceral fat and retained independent significance after adjusting for visceral fat (31). Further, in a review of 23 published studies of intervention strategies to promote loss of visceral adipose tissue, measured by magnetic resonance imaging or CT, Smith and Zachwieja (32) concluded that individuals with greater visceral fat mass, either through an increase in body weight or the propensity to store fat in the visceral depot, lose more visceral fat when adjusted to the loss of body fat, regardless of the intervention applied (caloric restriction, pharmacological therapy, or exercise) because the visceral adipocyte has a higher lipolytic rate also in the steady state. In addition, it has been emphasized that the endocrine abnormalities described in obesity, which involve steroid hormones, GH, and insulin, may actually result in abdominal depot fat accumulation. This might cause the metabolic syndrome in the susceptible individual (33, 34).

In this review I will evaluate the methods for assessment of abdominal fat content, from anthropometric indicators to imaging techniques, and their usefulness for predicting changes in visceral fat. The correlations of abdominal visceral fat with age, gender, total body fat, energy balance, adipose tissue lipoprotein lipase (LPL) and lipolytic activity, and genetic characteristics will be presented. The pathology of intraabdominal visceral fat without a specific endocrine disorder, considering the increase in intraabdominal fat content in nonobese and obese subjects and, on the other hand, the lipodystrophic syndromes with a reduction of visceral fat, will be analyzed. Finally, I will discuss the endocrine regulation of abdominal visceral fat, taking into consideration the factors expressed and released by adipose tissue and the hormones known to have a role in human obesity: cortisol, testosterone, estrogens, and GH.

II. Classification of Abdominal Fat

As described by Märin *et al.* (21), abdominal fat is composed of abdominal subcutaneous fat and intraabdominal fat, as clearly shown by CT and magnetic resonance imaging (MRI); intraabdominal adipose tissue is composed of visceral, or intraperitoneal, fat, mainly composed of omental and mesenteric fat and retroperitoneal fat masses by a delineation along the dorsal borderline of the intestines and the ventral surface of the kidney.

According to Abate *et al.* (30) the two intraabdominal compartments are separated on MRI using anatomical points, such as ascending and descending colon, and aorta and inferior vena cava; such a procedure has been validated in human cadavers (35). However, the lack of exact borderlines between these two depots on CT or MRI makes this subdivision only an approximation. Even a large error in the delineation between these two tissues would lead to the conclusion that, at least in men, the retroperitoneal fat mass is a minor part of intraabdominal adipose mass, comprising only approximately one fourth of visceral fat (21). On the other hand, the intraperitoneal and retroperitoneal adipose tissue masses measured after dissection in three cadavers were 61–71% and 29–33%, respectively, of the intraabdominal adipose tissue mass (35). Abate *et al.* (30) studied healthy middle-aged men with a wide range of adiposity and found that the retroperitoneal fat mass decreased from 42 to 31% of the intraabdominal adipose tissue mass, respectively, in lean and obese subjects. Märin *et al.* (21) have shown a stronger correlation for visceral than for retroperitoneal adipose mass with systemic metabolic variables, including plasma insulin, blood glucose levels, glucose disposal rate (euglycemic clamp), and systolic blood pressure. Similarly, Abate *et al.* (30) showed that while there was a significant and negative correlation with glucose disposal during an euglycemic hyperinsulinemic clamp, no such relationship was observed with the retroperitoneal fat mass and, as mentioned previously, the subcutaneous adipose tissue in the truncal region,

including thorax and abdomen, contributed more to insulin resistance than the adipose tissue elsewhere in the body. Thus, their investigation, as well as that of Goodpaster *et al.* (31), suggested that subcutaneous abdominal fat, as a component of central obesity, has as strong an association with insulin resistance as visceral fat and retained independent significance after adjusting for visceral fat.

III. Assessment of Abdominal Visceral Fat

A. Anthropometric indexes of abdominal visceral adipose tissue mass

1. WHR. The WHR is the most widely used index of regional adipose tissue distribution and is measured in a standing position. Waist circumference is defined as the minimal circumference measured at the navel, and the hip circumference is defined as the widest circumference measured at the hips and buttocks (36).

There is a well documented sex dimorphism in regional adipose tissue distribution (37). Indeed, despite the fact that women are usually more obese as a group than men, male subjects more frequently have significantly higher mean waist circumference and higher mean WHR in agreement with the greater propensity of men to accumulate excess fat within the abdominal cavity. Thus, the threshold values suggested by Pouliot *et al.* (38) of 0.85 for women and 0.95 for men are in agreement with those proposed in previous studies (39) and in our small series of normal men and women where the mean + 2 SD was 0.97 and 0.86, respectively (unpublished data). Since the WHR has been shown to be associated, albeit moderately, with the amount of abdominal visceral adipose tissue measured by CT or MRI [the "gold standards" for such determination (40–42)], this index has been widely used to investigate the relations between regional adipose tissue distribution and metabolic profile. Thus it was effective in predicting aberrations in glucose and insulin levels and also showed a strong correlation between plasma lipids and blood pressure (14). WHR predicted subsequent diabetes in men (13) and coronary heart disease in both men and women (8, 9) and was more predictive of these endpoints than either the BMI or a more complex procedure using the sum of multiple skinfold thicknesses. Its effects are independent of the overall level of obesity. However, Pouliot *et al.* (38), in a study using a large sample of men and women, showed that the use of WHR as a single anthropometric index of cardiovascular risk, as well as the use of critical threshold values indicated by their current data, is limited by the fact that, for a given WHR value, there may be large variations in the level of total body fat and in the level of abdominal visceral adipose tissue that are most likely to be associated with important variations in the metabolic profile. Thus, according to their data, the WHR determines the regional distribution of adipose tissue, which is relatively independent of the degree of obesity and appears less closely related to the amount of abdominal visceral adipose tissue. In this study (38), other simple anthropometric indexes were evaluated that appeared to be superior to the WHR in providing assessment of visceral obesity, waist circumference, and abdominal sagittal diameter (to be discussed below). Similar conclusions were reached by Sjöström *et al.* (43).

2. Waist circumference. Of the body circumferences, the measurement at the abdomen or "waist" is the most variable in term of its location or position, especially among obese and elderly persons. For example, the waist circumference is correctly measured at the level of the umbilicus, but in many obese individuals, the umbilicus may be directed downward because of the excessive curvatures of the abdominal wall.

Waist circumference measured at the midpoint between the lower border of the rib cage and the iliac crest has been reported to be more closely correlated with the level of abdominal visceral adipose tissue and associated metabolic variables than the WHR in both sexes (38, 42, 44, 45). According to Pouliot *et al.* (38), a waist circumference greater than 100 cm is most likely to be associated with disturbances in lipoprotein metabolism and in plasma glucose-insulin homeostasis (at least in French Canadians). The threshold value is similar in men and women in that for a given waist circumference, men and women had comparable levels of abdominal visceral adipose tissue. Thus, waist circumference, a convenient and simple measurement unrelated to height (46) and correlated with BMI and WHR (47), determines the extension of abdominal obesity, which appears closely linked to abdominal visceral adipose tissue deposition. Furthermore, while changes in waist girth reflect changes in risk factors for cardiovascular disease (48) and other forms of chronic disease, the risks vary in different populations; therefore, globally applicable cut-off points cannot be developed. For example, abdominal fatness has been shown to be less strongly associated with risk factors for cardiovascular disease and type 2 diabetes in black women than in white women (49). Risk factors such as total and HDL cholesterol were correlated with subcutaneous and abdominal fat areas by CT as well as their sum in healthy nonobese Asian Indians. On the other hand, while there was an association of visceral adiposity with insulin secretion during an oral glucose test in men, such was not found in women (50). In addition, it has been reported that visceral obesity is strongly related to coronary heart disease risk factors in nonobese Japanese-American men (51). Also, people of South Asian (Indian, Pakistani, and Bangladeshi) descent living in urban societies have a higher incidence of obesity complications than other ethnic groups (52). These complications are seen to be associated with abdominal fat distribution, which is markedly higher for a given level of BMI than in Europeans. Finally, although women have an almost equivalent absolute risk of coronary heart disease (CHD) to men at the same WHR (53, 54), they show increases in relative risk of CHD at lower waist circumferences than men. For example, in a random sample of 2,183 men and 2,689 women from the Netherlands, aged 20–59 yr (54), risk of obesity-associated metabolic complications was either increased or substantially increased in men with a waist girth of $\geq$ 94 and $\geq$ 102 cm, respectively. In women, the correspondent values were $\geq$ 80 and $\geq$ 88 cm, respectively. Thus, there is a need to develop sex-specific waist circumference cut-off points appropriate for different populations.

The studies by Ferland *et al.* (42) and Pouliot *et al.* (38)

revealed that the shared variance between waist circumference and visceral adipose tissue reached 75%, which suggests that waist girth by itself may be a useful variable for the crude assessment of visceral fat accumulation. Indeed, their results indicate that more than 90% of the variation in waist girth could be explained by differences in total body fatness and in visceral adipose tissue accumulation in both men and women.

Therefore, the waist circumference, and the abdominal sagittal diameter (as will be discussed below), are the anthropometric indexes preferred over the WHR to estimate the amount of abdominal visceral fat and related cardiovascular risk profile.

Using the equations for prediction, multiscan CT was used to determined visceral adipose tissue volume from the waist circumference in a sample of 17 males and 10 females with different degrees of obesity (43). The waist circumference explained 60% and 64% of the visceral adipose tissue volume variance in males and females, respectively, and the SE was 26% and 29%, respectively. The corresponding figures for the WHR were 26% and 69%, and 36% and 27%, respectively. Again, it was concluded that the WHR is a suboptimal predictor of visceral adipose tissue volume.

3. Abdominal sagittal diameter. Abdominal sagittal diameter is derived either from a CT abdominal scan (38) or by using a carpenter's spirit level placed over the abdomen perpendicular to the length axis of the trunk at the iliac crest level when the subject is placed on a firm examination table. The sagittal diameter is measured with a ruler as the vertical distance from the horizontal spirit level to the examination table after a normal expiration (43).

Kvist *et al.* (55) were the first to demonstrate that the sagittal diameter (measured on a CT scan) was closely related to the volume of visceral fat. The correlation of the sagittal diameter with visceral fat volume was 0.94 in 19 women and 0.92 in 24 men, the subjects presenting a wide range of BMI. The correlations between the waist circumference and visceral fat were, respectively, 0.85 and 0.88. These correlations are considerably higher than those observed between anthropometric variables and the visceral fat area measured at the level of the umbilicus in obese men and women (56). Ferland *et al.* (42) also observed markedly lower correlations in obese women. Després *et al.* (45), in a study of men covering a wide range of fatness, also observed higher correlations but there was not much difference between the visceral fat area and the correlations with the waist circumference ($r = 0.82$) and the sagittal diameter ($r = 0.85$). Busetto *et al.* (57) demonstrated that the waist girth was more closely related to the visceral fat area in nonobese compared with obese subjects. It is very likely, therefore, that the range of fatness in subjects studied greatly influences the magnitude of the correlations and perhaps also the comparison between the sagittal diameter and the waist circumference with regard to their utility in predicting intraabdominal fat. In addition, the distinction between studies that used only visceral fat area and those that calculated visceral fat volume from multiple scans may be important to make (58). Ross *et al.* (59) showed that correlations of the waist with the visceral fat area ($r = 0.65$) were weaker than those with the visceral fat volume ($r = 0.78$) in obese women.

A study from the Canadian group (38) conducted in a large group of males and females evaluated systematically the three anthropometric indexes and their association with abdominal visceral adipose and subcutaneous areas measured by CT (between the fourth and fifth lumbar vertebrae) and metabolic profile. As seen in Table 1, there was a strong association between waist girth and body fat mass, the slope of the regression line being steeper in women (data not shown). With relation to the abdominal visceral fat area, for a given waist circumference, men and women had similar levels and the slopes of the regression lines were not different between genders. Essentially similar results were observed with the abdominal sagittal diameter. However, in contrast with waist circumference, the slopes of regression of abdominal sagittal diameter to abdominal visceral fat area were significantly different between genders and were steeper in men (data not shown). Finally, it can be seen that the WHR was less strongly correlated with total body fat mass and abdominal visceral and subcutaneous areas than the other indexes. This study demonstrated that most of the variance in waist girth and abdominal sagittal diameter can be explained by variations in body fat mass and in abdominal visceral and subcutaneous adipose tissue areas ($0.85 \leq R^2 \leq 0.95$), whereas a lower proportion of the variance in the WHR could be explained by these adipose variables ($R^2 = 0.46$ and 0.60 in men and women, respectively). With relation to the metabolic variables related to cardiovascular risk (plasma triglycerides and high-density lipoprotein cholesterol levels, fasting and postglucose glucose and insulin levels), in women, the waist circumference and the abdominal sagittal diameter were more closely related to the metabolic variables than the WHR, whereas such differences were not apparent in men. They concluded that waist circumference values above approximately 100 cm, abdominal sagittal diameter values greater than 25 cm, and WHR values greater than 0.8 in women and 1.00 in men were likely to be associated with

TABLE 1. Correlations (r values) between the anthropometric indexes and body fat mass, abdominal visceral, and abdominal subcutaneous fat areas in 81 men and 70 women

	Body fat mass[a] (kg)		Abdominal visceral fat area[b] (cm^2)		Abdominal subcutaneous fat area[b] (cm^2)	
	Men	Women	Men	Women	Men	Women
Waist-to-hip ratio	0.70	0.55	0.71	0.67	0.68	0.47
Waist circumference (cm)	0.93	0.94	0.77	0.87	0.90	0.91
Abdominal sagittal diameter (cm)	0.87	0.95	0.80	0.87	0.86	0.95

[Derived from Ref. 38.]
[a] By underwater weighing.
[b] By computed tomography.

disturbances in lipoprotein metabolism and plasma insulin-glucose homeostasis, suggesting that the waist girth or the abdominal sagittal diameter, rather than the WHR, should be used as indexes of abdominal visceral adipose tissue deposition for the assessment of cardiovascular risk.

Correlations between sagittal diameter and waist circumference are usually quite high [*e.g.*, $r = 0.84$ in obese men and 0.76 in obese women (56)]. In an epidemiological study in men aged 18–55 yr the correlation between sagittal diameter and waist circumference was even higher at $r = 0.899$ (60). Although the sagittal supine diameter can be studied with relatively good precision (61), it is clear that this measurement requires appropriate equipment and skilled personnel. Since most people are measuring the WHR as an indicator of visceral fat, the focus should be switched to the waist girth alone without affecting the ranking of individuals with respect to visceral fat when based on the waist circumference compared with the sagittal diameter (58).

B. Imaging techniques

1. Computed tomography (CT). CT can be considered the gold standard not only for adipose tissue evaluation but also for multicompartment body measurement (61, 43). The reported error for the determination of total adipose tissue volume after performing 28 scans is 0.4%, which supports the high reproducibility of CT. The subcompartments of adipose tissue volume, visceral and subcutaneous adipose tissue, can be accurately measured with errors of 1.2 and 0.5%, respectively. Since the visceral fat volume has been determined from the visceral adipose tissue area of several scans, it is independent of individual visceral adipose tissue distributions; the precision error of this volume determination was reported to be in the order of 1% by Chowdhury *et al.* (61). In eight nonobese Swedish males evaluated by the multiscan CT technique, the volume of visceral abdominal adipose tissue in the intraperitoneal and retroperitoneal compartments was found to be 1.96 ± 1.13 and 0.78 ± 0.51 L (mean $\pm$ SD), respectively (62). It was found that the sagittal diameter (L4-L5) was a more specific predictor of visceral adipose tissue volume than waist circumference and WHR; the estimates based on sagittal diameter had errors in the order of 20%, while those based on visceral fat had only slightly lower errors (10–14%) (43). Using a multislice magnetic resonance protocol, Abate *et al.* (30) and Ross *et al.* (59, 63) found values for visceral adipose tissue and its subcompartments, particularly retroperitoneal fat, within the range found by Sjöstrom (62). In effect, in 13 lean males, Abate *et al.* (30) found 1.1 ± 0.5 and 0.8 ± 0.3 kg for intraperitoneal and retroperitoneal fat, respectively.

If only one scan is used to measure the visceral adipose tissue area, a strictly defined longitudinal level is very important since the average visceral adipose tissue area shifts if there is a change in position, even of a few centimeters. This, according to Sjöström *et al.* (43), implies that examinations at the so called umbilical level, as performed by many investigators, are not sufficiently exact since the umbilicus of obese subjects may be located at a lower position. Instead, the longitudinal level must be defined in a strict relation to the skeleton, usually between the L4 and L5 vertebrae. Kvist *et al.* (55) have found that visceral fat areas from a single scan in the L4-L5 region are highly correlated to the total visceral fat volume in both sexes.

To determine the visceral intraabdominal and subcutaneous abdominal areas, a simple CT (or MR) scan is taken either at the level of L4-L5 or the umbilicus, with an attenuation range of -30 to -190 Hounsfield units (64, 65). The subjects are examined in a supine position with their arms stretched above their heads. The choice to perform the scan at the level of the umbilicus was initially proposed by Borkan *et al.* (66), who found that at the level of the umbilicus there is the highest percentage of body fat, and it best allows differentiation of subcutaneous from intraabdominal fat. Subsequently, Tokunaga *et al.* (67) also suggested taking the measurement at the umbilicus. In addition to the recommendations of the Japanese investigators, studies from Korea (20) and from our clinic use the scan at the umbilicus.

Visceral fat is defined as intraabdominal fat bound by parietal peritoneum or transversalis fascia, excluding the vertebral column and the paraspinal muscles; subcutaneous fat is fat superficial to the abdominal and back muscles. Subcutaneous fat area is calculated by subtracting the intraabdominal fat area from the total fat area. The ratio of intraabdominal visceral fat (V) to the sc fat area (S)— V/S—as a relative index of intraabdominal fat accumulation, was shown to be strongly related with disorders of glucose and lipid metabolism in obese subjects, these metabolic parameters being significantly higher in the so-called visceral group (with a V/S ratio of ≥ 0.4) than in the subcutaneous group (with a V/S ratio of < 0.4) (17). The same authors (17) have found that glucose and lipid metabolism in the visceral group was disordered independent of sex, age, and BMI, with males having a higher V/S ratio than females and the individuals with high V/S ratios tending to be older than those with lower V/S ratios. In addition, visceral fat increases with age (68). Figure 1 shows cross-sectional abdominal areas obtained by CT at the level of the umbilicus in two women matched for the same BMI, who differed markedly in the accumulation of fat in the abdominal cavity but less so in the subcutaneous abdominal fat.

In obese subjects the level of the umbilicus can change from one patient to another, thus changing the visceral adipose tissue area; therefore, it is advisable that the scan area be defined in strict relation to the skeleton. Chowdhury *et al.* (69) showed greatly different average visceral adipose tissue areas in five different levels (from TH 8–9 to the sacroiliac joint) in nine men with body weight 114 ± 20 kg and age 44 ± 11 yr (mean $\pm$ SD). However, the values for abdominal cut-off points were related to increased cardiovascular risk (Table 2). Using the scan at the umbilicus as described by several investigators gave results similar to, although somewhat lower than, those reported using the L4-L5 level.

Regarding the relationship between the modifications in subcutaneous and visceral adipose tissue, with changes in body weight, it was shown that after severe weight loss, subcutaneous fat at the abdominal level is lost in greater proportion than visceral fat, but the mechanism of these differential changes in both compartments of abdominal fat is unknown, suggesting that visceral fat does not reflect nutritional status to the extent that sc fat does (70). In the

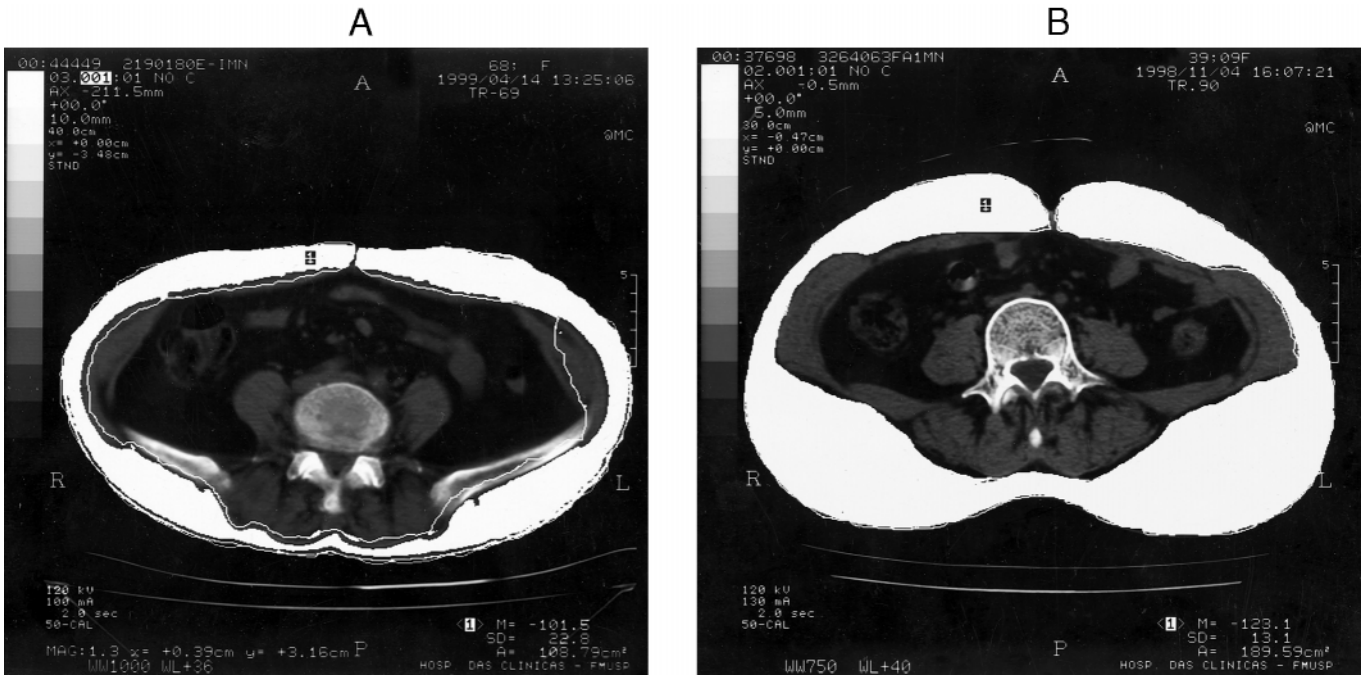

FIG. 1. Computed tomography showing cross-sectional abdominal areas at umbilicus level in two patients demonstrating variation in fat distribution. A, Visceral type (49-yr-old female, 23.1 of BMI, visceral fat area: 146 cm^2; subcutaneous fat area, 115 cm^2; V/S ratio, 1.27). B, Subcutaneous type (40-yr-old female, 24.0 of BMI, visceral fat area: 60 cm^2; subcutaneous fat area, 190 cm^2; V/S ratio, 0.31).

TABLE 2. Abdominal visceral adipose tissue area cut-off points related to increased cardiovascular risk

Study	Computed tomography		Magnetic resonance imaging	Sex		Obesity		Abdominal visceral fat area (cm^2)
	Umbilicus	L_4–L_5		Male	Female	Present	Absent	
Desprès and Lamarche (73)		+		+	+	+	+	≥130
Hunter *et al.* (74)		+		+		+	+	≥131
Williams *et al.* (75)		+			+	+	+	≥110
Anderson *et al.* (77)		+	+	+	+	+	+	≥132[a]
Matsuzawa *et al.* (78)	+			+			+	≥133[b]
Saito *et al.* (79)	+			+		+	+	≥100[c]
					+	+	+	≥90[c]
Lottenberg *et al.* (80)	+			+	+	+	+	≥107

[a] Chinese type 2 diabetics.
[b] Nonobese coronary heart disease Japanese patients.
[c] Japanese subjects.

same way, published data suggest that, at least in relative terms, visceral fat increases less than subcutaneous fat with increased body weight (71). However, because the amount of subcutaneous abdominal fat is calculated indirectly, it is likely that significant measurement error could be introduced (32).

Regarding the reproducibility of CT measurement of visceral adipose tissue area, Thaete *et al.* (72) evaluated duplicate cross-sectional CT scans of the abdomen at the L4 level in 16 healthy premenopausal women, who ranged from lean to obese. The duplication occurred after the initial scan; the subjects were repositioned before repeat scanning. Excellent reproducibility was shown by a high correlation between duplicate measurements ($r = 0.99$) and by small precision errors: 1.2% of the mean value for total adipose tissue cross-sectional area, 1.9% for subcutaneous adipose tissue area, and 3.9% for visceral adipose area.

As indicated in the *Introduction*, individuals with a high accumulation of visceral abdominal fat, as shown by CT scans, had an increased risk for development of type 2 diabetes, dyslipidemia, and coronary heart disease. Table 2 shows the thresholds above which metabolic complications would be more likely to be observed in visceral adipose tissue areas. Desprès and Lamarche (73), Hunter *et al.* (74), and Williams *et al.* (75) studied men and/or women with a wide range of body weight. They found that a value above 110 cm^2 was associated with an increased risk of coronary heart disease in pre and postmenopausal women (75); the same group (74) found that males with abdominal visceral fat cross-section areas measuring more than 131 cm^2 were clearly at an increased risk for coronary disease. On the other hand, Desprès and Lamarche (73) found that in both men and women a value of 100 cm^2 was associated with significant alterations in cardiovascular disease risk profile and that a further deterioration of the metabolic profile was observed when values greater than 130 cm^2 of visceral adipose tissue

were reached. From the same center, Lemieux *et al.* (76) determined in a sample of 213 men and 190 women the threshold values of the anthropometric parameters corresponding to an accumulation of visceral adipose tissue of 130 cm^2: a waist girth of approximately 95 cm in both sexes, sagittal diameters of 22.8 cm in men and 25.2 cm in women, and WHR values of 0.94 in men and 0.88 in women. In both sexes, threshold values of those anthropometric indexes were generally lower in subjects who were $\geq$ 40 yr old than in younger individuals. It was concluded that waist circumference was a more convenient anthropometric correlate to visceral adipose tissue because its threshold values did not appear to be influenced by sex or by the degree of obesity. Anderson *et al.* (77) examined the relationship between visceral abdominal fat area by MRI and cardiovascular risk factors in Chinese type 2 diabetes and found a threshold value of 132 cm^2.

The most extensive studies using a single CT scan at umbilical level was done by Matsuzawa and colleagues (17, 78). As indicated above, the data were expressed by the ratio of visceral fat area (V)/subcutaneous fat area (S), the cut-off point for the risk factors for cardiovascular disease, particularly those related to glucose and lipid metabolism and hypertension, being > 0.4. However, they did not present the raw data on visceral and subcutaneous areas but only their ratios, thus precluding their inclusion in Table 2. In a study of fat distribution in 29 nonobese coronary heart disease patients in comparison with 21 nonobese controls in which the data on abdominal visceral and subcutaneous abdominal fat areas were available, 34% of the patients had visceral areas above the maximal level found in the controls (132 cm^2), there being a significant difference ($P < 0.01$) between the two groups while no differences were found in the subcutaneous abdominal areas (78). In another study, performed in Japan by Saito *et al.* (79) in nonobese and obese males and females, fat areas at the umbilicus level as determined by CT had threshold values $\geq$ 100 for men and $\geq$ 90 for women and the V/S was also > 0.4. Lottenberg *et al.* (80) indicated a threshold value of $\geq$ 107 cm^2 for abdominal visceral fat area in a group of obese individuals.

2. Magnetic resonance imaging (MRI). MRI provided results similar to CT without exposure to ionizing radiation, the main problem with CT multislice measurements. It demonstrated good reproducibility for total and visceral adipose tissue volumes (63), which were slightly lower than previously reported using CT (55), although the percent contribution of visceral to total adipose tissue volume was similar (18 *vs.* 20%). Subcutaneous adipose tissue and visceral fat areas at the L4-L5 level determined in 27 healthy men by MRI were 252.8 $\pm$ 132.9 and 117.9 $\pm$ 62.1 cm^2 (mean $\pm$ SD), respectively, and the differences between two measurements for a single scan ranged from 1.4 to 4.2% (63). These areas were highly predictive of the corresponding volume measurements computed from the 41-scan MRI, confirming the CT studies of Kvist *et al.* (55), who made similar observations in both male and female subjects.

Two studies have compared estimates of subcutaneous and visceral adipose tissue by CT and MRI. Comparison between MRI and CT in seven subjects showed a high degree of agreement in measurement of total subcutaneous adipose tissue area but not visceral adipose tissue area (81). Moreover, it has been shown (82) that MRI when compared with CT overestimates subcutaneous adipose tissue (+8%) and visceral adipose tissue (+22%). As already mentioned, MRI has been validated in three cadavers, confirming its accuracy (35).

3. Ultrasound (US). US subcutaneous and intraabdominal thicknesses, the latter corresponding to the distance between abdominal muscle and aorta, were measured 5 cm from the umbilicus on the xipho-umbilical line with a 7.5-MHz probe for subcutaneous adipose tissue and a 3.5-MHz probe for intraabdominal fat (71). The intraindividual reproducibility of US measurements was very high both for intraabdominal and subcutaneous thickness as well as for interoperators (83, 84).

Several studies demonstrated a highly significant correlation between the intraabdominal adipose tissue determined by CT and by US. A decade ago, Armellini *et al.* (85) found a reasonable correlation ($r = 0.67$) of intraabdominal US measurements with CT at the L4-L5 level. In a more recent study, Tornaghi *et al.* (84) found a highly significant correlation between intraabdominal thickness and CT visceral adipose tissue area ($r = 0.89–0.91$) and Radominski (86), at The Hospital das Clinicas of São Paulo, Brazil, also observed in 24 subjects an excellent correlation between ultrasonography and CT ($r = 0.79$ for sc abdominal thickness and $r = 0.84$ for visceral adipose tissue), again indicating that US intraabdominal thickness is an excellent predictor of visceral abdominal adipose tissue (83, 87, 88). Furthermore, the correlation between abdominal sagittal diameter and CT in Radominski's study was lower than that observed between US and CT. In a cross-validation study, intraabdominal adipose tissue measured by CT was significantly correlated with intraabdominal adipose tissue predicted from an equation using primarily US intraabdominal thickness ($r = 0.84$) (88).

In a study of 191 men (C. C. Leite, D. Matsuda, B. L. Wajchenberg, G. G. Cerri, and A. Halpern, unpublished data), in which 53.5% presented some of the risk factors for cardiovascular disease, it was shown that intraabdominal thickness by US measurements was a better predictor of cardiovascular risk [Odds ratio (OR) = 2.27 (95% CI = 1.05–4.8)] than the anthropometric measurements (waist circumference [OR=1.53 (95% CI=0.47–5.0)] and sagittal diameter [OR = 0.8 (95% CI = 0.3–1.9)]). The cut-off points for moderate risk (2 or more of the following: total serum cholesterol > 200 and < 240 mg/dl; triglycerides > 200 mg/dl + HDL cholesterol > 35 and < 45 mg/dl; systolic > 140 and diastolic blood pressure > 90 mm Hg) and high risk (2 or more of the factors: cholesterol > 240 mg/dl; HDL cholesterol < 35 mg/dl; triglycerides > 200 + HDL < 35 mg/dl; plasma glucose > 126 mg/dl and systolic > 140 and diastolic blood pressure > 90 mm Hg) were 7 cm (sensitivity of 72% and specificity of 53%) and 9 cm (53% sensitivity and 83% specificity), respectively.

In obese women, after a 6-kg weight loss, a significant decrease was found in intraabdominal fat but not in subcutaneous adipose tissue, as determined by both CT and US

(87). There was also a significant correlation between changes in intraabdominal adipose tissue using both techniques, indicating that US can be used in the evaluation of body fat distribution modifications during weight loss. Further, by subdividing a group of 119 obese women with a wide range of BMIs into tertiles of intraabdominal adipose tissue as evaluated by CT (<114, 114–170, >170 cm^2), Armellini *et al.* (83) observed that intraabdominal US measurements were significantly different in the intraabdominal CT tertiles (16 ± 10, 32 ± 13, 50 ± 22 mm, respectively; $P < 0.001$) while neither sagittal diameter nor WHR was able to distinguish between the two >114 cm^2 groups. This is another confirmation of the reliability of the US intraabdominal determinations.

IV. Correlations of Abdominal Visceral Fat

A. Age and gender

The amount of visceral fat increases with age in both genders, and this increase is present in normal weight (BMI, 18.5 to 24.9) as well as in overweight (BMI, 25 to 29.9) and obese subjects (BMI > 30 kg/m^2) but more so in men than in women (7, 68, 90). In a study of 130 subjects (62 males and 68 females with a wide range of age and weight), Enzi *et al.* (68) found that in young females, either lean or obese, the subcutaneous abdominal fat area was predominant over abdominal visceral fat, both measured by CT at the upper renal pole. This fat topography was retained in young and middle-aged females up to about 60 yr of age, at which point there was a change to an android type of fat distribution. This age-related redistribution of fat is due to an absolute as well as relative increment in visceral fat depots, particularly in obese women, which could be related to an increase in androgenic activity in postmenopausal subjects. On the other hand, they showed that males at any age tend to accumulate fat at the visceral depot, increasing with age and BMI increase. In the male, a close linear correlation between age and visceral fat volume was shown, suggesting that visceral fat increased continuously with age (78). Although this correlation was also present in women, the slope was very gentle in the premenopausal condition. It became steeper in postmenopausal subjects, almost the same as in males (78). Further, Enzi *et al.* (68) found that 7.3% of the females in their study had an android type of body fat topography and 6.5% of the males had a gynoid type of fat distribution.

From the published data (68, 90), it can be concluded that both subcutaneous and visceral abdominal fat increase with increasing weight in both sexes but while abdominal subcutaneous adipose tissue decreases after the age of 50 yr in obese men, it increases in women up to the age of 60–70 yr, at which point it starts to decline (71). Fowler *et al.* (91) evaluated total and subcutaneous adipose tissue with a multislice MRI in obese and lean women and found that the main difference between them was in the percentage of subcutaneous fat in the abdomen, less than in the thighs in lean, while no significant differences between both sites of subcutaneous adipose tissue were observed in obese individuals. Finally, as previously indicated, visceral fat is more sensitive to weight reduction than subcutaneous adipose tissue because omental and mesenteric adipocytes, the major components of visceral abdominal fat, have been shown to be more metabolically active and sensitive to lipolysis (92).

Lemieux *et al.* (93) have indicated that the gender difference in visceral adipose tissue accumulation was an important factor in explaining the gender differences in cardiovascular risk profile. In addition, the adjustment for differences in visceral fat between men and women eliminated most of the sex differences in cardiovascular risk factors. There is evidence supporting the notion that abdominal visceral fat accumulation is an important correlate of the features of the insulin-resistant syndrome (23, 24, 29) but this should not be interpreted as supporting the notion of a cause and effect relationship between these variables (27). This subject will be discussed later on.

B. Total body fat

The correlations of abdominal visceral fat mass evaluated by CT or MRI scans with total body fat range from 0.4 to 0.8, with higher values obtained when a large range of fatness, from lean to obese, is present in the population (40, 42, 44, 45, 64). They tend to be lower in the lean and normal weight subjects than in the obese (44). As indicated by Bouchard *et al.* (7) it is important to recognize that individual differences in abdominal visceral fat remains considerable even when subjects with relatively similar BMI and percent body fat are investigated. When they examined the relationship of total body fat mass to visceral adipose tissue accumulation in men and in premenopausal women, Lemieux *et al.* (94) reported that for a given amount of total body fat men had about twice the amount of visceral adipose tissue than what is found in premenopausal women. Furthermore, the relationship of visceral adipose tissue to metabolic complications was found to be independent of concomitant variation in total body fat, and it was concluded that the assessment of cardiovascular risk in obese patients solely from the measurement of body weight or of total body fatness may be completely misleading (19, 22, 36, 95). Indeed, it appears that only the subgroup of obese individuals characterized by a high accumulation of visceral adipose fat show the complications predictive of type 2 diabetes and cardiovascular disease (27). On the other hand, after adjustment for total body fat, Abate *et al.* (30) have shown that intraperitoneal adipose tissue lost its significant correlation to the parameters of insulin resistance during an hyperinsulinemic euglycemic clamp, failing to demonstrate that intraperitoneal (visceral) fat uniquely enhances insulin resistance and thus the associated risks.

C. Energy balance

Intraabdominal visceral fat is associated with an increase in energy intake but this is not an absolute requirement. Positive energy balance is a strong determinant of truncal-abdominal fat as shown by Bouchard and colleagues (96) in overfeeding experiments in identical twins. The correlations between gains in body weight or total fat mass with those in subcutaneous fat on the trunk reached about 0.7 in their 100-day overfeeding study in 12 pairs of male identical twins. In contrast, these correlations attained only 0.3 with the gains

in abdominal visceral fat, corresponding to a common variance of less than 10% (7, 96). Thus, positive energy balance does not appear to be a strong determinant of abdominal visceral fat as is the case with other body fat phenotypes (7). In effect, as discussed in the CT section of imaging techniques for evaluation of intraabdominal visceral fat, some investigators (70, 71) have shown that either when the subjects lose or increase their weight, particularly females, visceral fat is lost or gained, respectively, less than subcutaneous fat at the abdominal level. However, at variance from these data, Zamboni *et al.* (97) have shown in premenopausal women that after weight loss, on a very low energy intake from 2 weeks to 3 months, visceral fat decreased more than subcutaneous fat, confirming (according to these authors) that visceral fat is more sensitive to weight reduction because omental and mesenteric adipocytes have been shown to be more metabolically active and sensitive to lipolysis (92). Similarly, as already mentioned, Smith and Zachwieja (32) noted that all forms of weight loss affect visceral fat more than subcutaneous fat (percentage wise), and there was a gender difference, with men appearing to lose more visceral fat than women for any given weight loss. This subject will be discussed later on.

D. Adipose tissue LPL activity

LPL activity, being related to the liberation of the lipolytic products [from chylomicra and very-low-density lipoproteins (VLDL)] to the adipocytes for deposit as triglycerides, is a key regulator of fat accumulation in various adipose areas, since human adipose tissue derives most of its lipid for storage from circulating triglycerides. However, adipocytes can synthesize lipid *de novo* if the need arises, as in patients with LPL deficiency (98).

It was demonstrated in men with a wide variation of body fat that the uptake of labeled triglycerides was higher in omental than in subcutaneous abdominal adipose tissue, amounting to as much as approximately 50% more in omental than in abdominal subcutaneous depots; however, this did not correlate with LPL activity in the tissue (21). This suggests that other factors may be as important as LPL for the regulation of triglyceride uptake *in vivo* in adipose tissue (21), such as the "acylation stimulating protein" (ASP), a strong stimulator of FFA reesterification and triglyceride synthesis in human adipose tissue that has an insulin-like effect and thus possibly plays a role in initiating and maintaining the obese state. According to Sniderman *et al.* (99), as fatty acids are being liberated from triglycerides as a result of LPL, ASP is also generated to ensure that the rate of triglyceride synthesis within adipocytes is sufficiently rapid that fatty acids in the microcirculation will not increase unduly, allowing the rapid hydrolysis of triglyceride-rich lipoproteins to continue and, consequently, rapid triglyceride clearance to occur. The increase of visceral fat masses with increasing total body fat was explained by an increase of fat cell size only up to a certain adipocyte weight. However, with further enlargement of intraabdominal fat masses with severe obesity, the number of adipocytes seems to be elevated (100, 101). In women, but not in men, omental adipose tissue has smaller adipocytes and lower LPL activity than subcutaneous fat depots since variations in LPL activity parallel differences in fat cell size (7). When adipocytes enlarge in relation to a gain in body weight, the activity of LPL increases in parallel, possibly as a consequence of obesity-related hyperinsulinism. The higher basal activity of adipose tissue LPL in obesity is accompanied by a lower increment after acute hyperinsulinemia (102). Lipid accumulation is favored in the femoral region of premenopausal women in comparison with men (103). In the latter, LPL activity as well as the LPL mRNA levels were greater in the abdominal than in gluteal fat cells, while the opposite was observed in women, suggesting that regional variation of gene expression and posttranslational modification of LPL could potentially account for the differences between genders in fat distribution (103). With progressive obesity, adipose tissue LPL is increased in the depots of fat in parallel with serum insulin. However, when obese subjects lost weight and became less hyperinsulinemic, adipose LPL increased further and the patients who were most obese showed the largest increase in LPL, suggesting that very obese patients are most likely to have abnormal LPL regulation, independent of the influence of insulin. This probably indicates that adipose tissue LPL activity may represent an adipocyte "set point" that is intended to limit adipocyte shrinkage induced by a hypocaloric diet (98). In response to feeding, the increase in LPL is, as indicated, due to posttranslational changes in the LPL enzyme. However, the increased LPL after weight loss involved an increase in LPL mRNA levels, followed by parallel increases in LPL protein and activity (104). Because the response to weight loss occurred via a different cellular mechanism, it is probably controlled by factors different from the day-to-day regulatory forces. In addition, because the very obese patients demonstrated a larger increase in LPL with weight loss than the less obese patients, these data suggest a genetic regulation of LPL that is most operative in the very obese (98). The role of sex steroids, glucocorticoids, and catecholamines in the regulation of adipose tissue LPL activity in various fat depots will be discussed in the section on hormonal regulation of abdominal visceral fat.

E. Adipose tissue lipolytic activity

Lipid mobilization and the release of FFA and glycerol are modulated by the sympathetic nervous system. Catecholamines are the most potent regulators of lipolysis in human adipocytes through stimulatory β_1- and β_2-adrenoreceptors or inhibitory α2-adrenoreceptors (105). A gene that codes for a third stimulatory β-adrenoreceptor, β_3-adrenoreceptor, is functionally active principally in omental adipocytes (106) but also present in mammary fat and subcutaneous fat *in vivo* (107). The main systems involved in the inhibitory control of lipolysis are insulin/insulin receptor and adenosine/adenosine receptor (102).

Regional differences in catecholamine-induced lipolysis and sensitivity to insulin's antilipolytic effects have been extensively described in *in vitro* studies. In both genders and independently of the degree of obesity, femoral and gluteal fat cells exhibit a lower lipolytic response to catecholamines than subcutaneous abdominal adipocytes, the latter showing both increased β_1- and β_2-adrenoreceptor density and sen-

sitivity and reduced α2-adrenoreceptor affinity and number (Refs. 7, 102; Table 3). Abdominal visceral adipocytes, compared with subcutaneous abdominal or femoral adipose cells, are more sensitive to catecholamine-induced lipolysis, equally (or slightly less) sensitive to both α2- and adenosine receptor-dependent inhibition of lipolysis, and less sensitive to insulin's antilipolytic effects. The increased sensitivity to catecholamine-induced lipolysis in omental fat in nonobese individuals is paralleled by an increase in the amount of β_1- and β_2-receptors, with normal receptor affinity and normal lipolytic action of agonists acting at postadrenoreceptor steps in the lipolytic cascade (108, 109); this is associated with enhanced β_3-adrenoreceptor sensitivity, which usually reflect changes in receptor number in comparison with subcutaneous adipocytes (110, 111).

Adipocytes from obese subjects generally show increased lipolytic responses to catecholamines, irrespective of the region from which they are obtained, and enhanced lipolysis in abdominal compared with gluteo-femoral fat (21, 101). The antilipolytic effect is also reduced *in vitro* in obesity, both in omental and subcutaneous adipocytes (112). The typical features of visceral fat, *e.g.*, increased sensitivity to the lipolytic action of catecholamines and reduced sensitivity to the antilipolytic effect of insulin, are also preserved in obesity (21, 113, 114).

An increased β_3-adrenoreceptor sensitivity to catecholamine stimulation may lead to an increased delivery of FFA into the portal venous system, with several possible effects on liver metabolism. These include glucose production, VLDL secretion, and interference with hepatic clearance of insulin (115), resulting in dyslipoproteinemia, glucose intolerance, and hyperisulinemia.

Lönnqvist *et al.* (116) investigated sex differences in visceral fat mobilization in obese males and females matched for BMI and age who were undergoing elective surgery. They observed that males had a higher fat cell volume with no sex differences in the lipolytic sensitivity to β_1- and β_2-adrenoreceptor-specific agonists or in the antilipolytic effect of insulin. However, the lipolytic β_3-adrenoreceptor sensitivity was 12 times higher in men, and the antilipolytic α2-adrenoreceptor sensitivity was 17 times lower in men. It was concluded that in obesity, the catecholamine-induced rate of FFA mobilization from visceral fat to the portal venous system is higher in men than women. This phenomenon is partly due to a larger fat cell volume, a decrease in the function of α2-adrenoceptors, and an increase in the function of β_3-adrenoreceptors. These factors may contribute to gender-specific differences observed in the metabolic disturbances accompanied by obesity, *i.e.*, males have higher abdominal sagittal diameter, blood pressure, plasma insulin, glucose, and triglyceride, and lower HDL cholesterol than females.

TABLE 3. Comparison of lipolysis, antilipolysis, and lipogenesis in omental and subcutaneous fat in nonobese and obese individuals

Omental fat		Subcutaneous fat	
Nonobese[a]	Obese[b]	Nonobese	Obese[c]
Lipolysis		Lipolysis	
1. β_1: ↑	β_1: =	1. β_1: ↓	β_1: =
2. β_2: ↑	β_2: =	2. β_2: ↓	β_2: ↓
3. β_3: ↑	β_3: ↑ ↑ ↑	3. β_3: 0	β_3: 0
Antilipolysis		Antilipolysis	
1. Adenosine: =	Adenosine: =	1. Adenosine: =	Adenosine: =
2. α_2: =	α_2: ↓	2. α_2: =	α_2: =
3. Insulin: ↓	Insulin: ↓ ↓	3. Insulin: ↑	Insulin: ↓
Lipogenesis		Lipogenesis	
1. LPL: ↓	LPL: ↑	1. LPL: ↑	LPL: ↑ ↑
2. Insulin: ↓	Insulin: ↓ ↓	2. Insulin: ↑	Insulin: ↓

[Derived from Ref. 102.]
[a] Omental *vs.* subcutaneous nonobese.
[b] Omental obese *vs.* omental nonobese.
[c] Subcutaneous obese *vs.* subcutaneous nonobese.

F. Adipose tissue receptors

1. Glucocorticoid receptors. Glucocorticoid receptors, one of the most important receptors for human adipose tissue function, are involved in metabolic regulation and distribution of body fat under normal as well as pathophysiological conditions. Glucocorticoid receptors in adipose tissue show a regional variation in density with elevated concentrations in visceral adipose tissue (117). With exposure to high concentrations of cortisol, such as in Cushing's syndrome, the density of glucocorticoid receptors is down-regulated; however, the differences in glucocorticoid receptor density between adipose tissues remain proportionally similar, but on a lower level, with visceral glucocorticoid receptor density remaining higher than subcutaneous adipose tissue (34). In spite of the lower receptor density, the elevated cortisol secretion results in clearly increased net effects of cortisol.

2. Androgen and estrogen receptors. Adipocytes have specific receptors for androgens, with a higher density in visceral fat cells than in adipocytes isolated from subcutaneous fat. Unlike most hormones, testosterone induces an increase in the number of androgen receptors after exposure to fat cells (118), thereby affecting lipid mobilization. This is more apparent in visceral fat (omental, mesenteric, and retroperitoneal) because of higher density of adipocytes and androgen receptors, in addition to other factors (34). However, at variance with the effects of testosterone, dihydrotestosterone treatment does not influence lipid mobilization (118). In females, there is an association between visceral fat accumulation and hyperandrogenicity, despite the documented effects of testosterone on lipid mobilization and the expected decrease in visceral fat depots. The observation that visceral fat accumulation occurs only in female-to-male transsexuals after oophorectomy (119) suggests that the remaining estrogen production before oophorectomy was protective (120). The androgen receptor in female adipose tissue seems to have the same characteristics as that found in male adipose tissue. However, estrogen treatment down-regulates the density of this receptor, which might be a mechanism whereby estrogen protects adipose tissue from androgen effects. Estrogen by itself seems to protect postmenopausal women receiving replacement therapy from visceral fat accumulation (121). Estrogen receptors are expressed in human adipose tissue (122) and show a regional variation of density, but whether the quantity of these receptors is of physiological importance has not been clearly established (34).

With regard to progesterone, adipose cells seem to lack binding sites and mRNA for progesterone receptors, indi-

cating that progesterone acts through glucocorticoid receptors (123).

3. GH receptors. While it is well established that GH has specific and receptor-mediated effects in adipose tissue of experimental animals, the importance of GH receptors in human adipose tissue is not fully elucidated at present although the available data indicate a functional role. However, GH is clearly involved in the regulation of visceral fat mass in humans. Acromegaly, a state of GH excess, is associated with decreased visceral fat while in GH deficiency there is an increase in visceral fat and in adults with GH deficiency, recombinant human GH replacement therapy results in adipose tissue redistribution from visceral to subcutaneous locations; however, the regulation of adipose tissue metabolism requires synergism with steroid hormones (34). A direct demonstration of a regulation of the GH receptor in human fat cells has not yet been performed (124).

4. Thyroid hormone receptors. Thyroid hormones have multiple catabolic effects on fat cells as a result of interactions with the adrenergic receptor signal transduction system, and most of these interactions are also present in human fat cells (125). There are data regarding the characterization of the nuclear T_3 receptor in human fat cells (126). Although receptor regulation has not yet been demonstrated, there is little doubt that the thyroid hormone receptors are important for the function of human adipose tissue (125). Further, no data are available on the correlation between visceral fat mass and thyroid hormone levels.

5. Adenosine receptors. Adenosine behaves as a potent antilipolytic and vasodilator agent and can be considered as an autocrine regulator of both lipolysis and insulin sensitivity in human adipose tissue. Site differences in ambient adenosine concentration, perhaps controlled by blood flow, may also modulate adipose tissue metabolism (7). Adenosine content is higher in omental than in abdominal subcutaneous adipose tissue, but the receptor-dependent inhibition of lipolysis is, as indicated before (102), less pronounced in the former than in the latter depot (127). However, despite strong antilipolytic effect of adenosine analogs, human adipocytes contain few adenosine type A_1 receptors, regardless of the fat depot considered (128).

According to Arner (124), the α2-, β_1 -, β_2-, and β_3-adrenoreceptors and receptors for insulin, adenosine, and glucocorticoids, as well as for PGE_2, a potent antilipolytic agent with high affinity receptors identified in adipocytes (129), have a major functional role, as shown by relevant biological receptor-mediated effects, the presence of a receptor molecule, and receptor regulation. The receptors for GH, thyroid hormones, estrogen, and testosterone, as well as for acetylcholine and TSH, probably have an important functional role but complete evidence, indicated in the previous group of receptors, is not present so far; however, there is little doubt of a regulatory role.

G. Genetic characteristics

1. Genetic epidemiology: heritability and segregation analysis. Studies performed in individuals from families of French descent living in Quebec City [Quebec Family Study (QFS)] allowed the estimation of the fraction of the phenotypic variance that could be attributed to the genetic and environmental factors among the obesity phenotypes or in the distribution of the adipose tissue, taking into account the BMI and amount of subcutaneous fat (by the sum of the measurement of skinfolds in six different sites), lean body mass, fat mass, percentage of fat derived from underwater weighing, and visceral fat by CT (130, 131). While the genetic effect was no more than 5% for the BMI and subcutaneous fat, it was 25% for the fat mass and percentage of fat and 56% for the visceral fat area after adjustment for age, sex, and total fat mass (130). A similar level of heritability (48%) was observed by the same group of investigators in another family study (HERITAGE Family Study) (132). The residual variance corresponded to environmental factors, but some factors (cultural, nongenetic) could be transmitted from parents to descendents and sometimes were confounded by genetic effects (131).

Segregation analysis studies have recently concluded that visceral fat is similarly influenced by a gene with a major effect in the QFS and HERITAGE families (133, 134). In the QFS families, the gene with the major effect, with an autosomic recessive transmission, corresponded to 51% of the variance for the visceral fat and the polygenic effects contributing to 21% of the variance. However, after adjustment of the visceral adipose tissue for the fat mass, the effect of the gene with the major effect was not more compatible with a mendelian transmission. These results suggested the presence of a pleiotropism: the gene with the major effect, identified by the fat mass (135), could similarly influence the amount of visceral fat (133). Similar results were obtained with the same type of analysis in the HERITAGE cohort (134).

To test the hypothesis of a genetic pleiotropism, Rice *et al.* (136) tried to determine whether, in addition to the genetic effects specific for the fat mass and visceral fat, there would be common genetic effects for the two phenotypes. The results of this study (Fig. 2) indicate that fat mass and visceral fat are influenced by genetic (G_1 and G_2) and environmental effects (E_1 and E_2), which are unique to them and the heritability of the two phenotypes is approximately 25 and 55%, respectively. Figure 2 also indicates that the common variance for the two phenotypes within the QFS cohort is about 43%. This phenotypic covariation is characterized by familial resemblances and the existence of common genetic factors for the two phenotypes (G_3) explaining their 30% covariation. These results have confirmed the presence of a genetic pleiomorphism and suggested the presence of genes affecting simultaneously the amounts of fat mass and visceral abdominal fat.

The interactions of the effects of genotype and environment evaluated in monozygotic twins, when the energy balance is manipulated, indicated that even though there were large interindividual differences in the response to excess or negative energy balance, there was a significant within-pair resemblance in response (96, 137). In effect, in response to overfeeding, there was at least 3 times more variance in response between pairs than within pairs for the gains in body weight, fat mass, and fat-free mass (96). In relation to the response to the negative energetic balance, at least 7 times

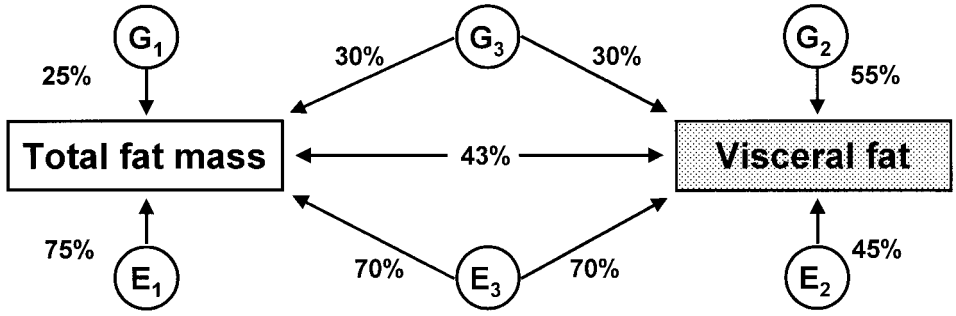

FIG. 2. Schematic representation of the genetic effects on total fat mass and visceral fat (adjusted for the fat mass) and on the co-variation between the two phenotypes (Quebec Family Study, 1996). G_1 and G_2 represent the genetic effects specific for the total fat mass and visceral fat, respectively. E_1 and E_2 represent the specific effects of the environment on total fat mass and visceral fat, respectively. G_3 and E_3 indicate the genetic and environment effects common to both phenotypes. [Reprinted with permission from L. Pérusse *et al.*: *Médecine/Sciences* 14:914–924, 1998 (131).]

more variation was observed in response between pairs than within members of the same pair of twins, with respect to the same variables (137). This intrapair similarity in the response to either excess or deficient energy balance is also observed in relation to the abdominal visceral fat (131). Thus, the interaction between genotype and environment is important to consider in the study of the genetics of obesity since the propensity to fat accumulation is influenced by the genetic characteristics of the subject.

2. Molecular genetics: association and linkage studies. Several candidate genes as well as random genetic markers were found to be associated with obesity as well as body fat and fat distribution in humans. The current human obesity gene map, based on results from animal and human studies, indicates that all chromosomes, with the exception of the Y chromosome, include genes or loci potentially involved in the etiology of obesity (138). Initial findings from the QFS showed that significant but marginal associations with body fat were found with LPL (139) and the α2-subunit of the sodium-potassium ATPase genes (140). The Trp64Arg mutation of the β_3-adrenergic receptor gene (β_3 AR), prevalent in some ethnic groups, is associated with visceral obesity and insulin resistance in Finns (141) as well as increased capacity to gain weight (142). This mutation was also shown to be associated with abdominal visceral obesity in Japanese subjects, with lower triglycerides in the Trp64Arg homozygotes but not heterozygotes (143). It has been suggested that those with the mutation may describe a subset of subjects characterized by decreased lipolysis in visceral adipose tissue. On the other hand, Vohl *et al.* (144) found that triglyceride levels were positively correlated with visceral obesity and hyperinsulinemia only in the subjects homozygous for the presence of the LPL *Hin*dIII polymorphism. Previously, it was reported by the same group that apo-B-100 gene *Eco*R-1 polymorphism appeared to modulate the magnitude of the dyslipidemia generally found in the insulin-resistant state linked with visceral obesity (145). These studies are a demonstration of a significant interaction between visceral obesity and a polymorphism for a gene playing an important role in lipoprotein metabolism.

When the genes related to the hormonal regulation of body fat distribution studied in the QFS families (sex hormone-binding globulin, 3β-hydroxysteroid dehydrogenase, and glucocorticoid receptor genes) were considered along with the knowledge that body fat distribution is influenced by nonpathological variations in the responsiveness to cortisol, it was shown that the less frequent 4.5-kb allele detected with the *Bcl*I restriction enzyme at the glucocorticoid receptor gene locus was associated with higher abdominal visceral fat area independently of total body fat mass. However, the association with abdominal visceral fat area was seen only in subjects of the lower tertile of the percent body fat level. In these subjects, the polymorphism was found to account for 41% and 35%, in men and women, respectively, of the total variance in abdominal visceral fat area. The consistent association between the glucocorticoid receptor polymorphism detected with *Bcl*I and abdominal visceral fat area suggested that this gene or a locus in linkage disequilibrium with the *Bcl*I restriction site may contribute to the accumulation of abdominal visceral adipose tissue (146).

With respect to the linkage studies, only a few studies of body fat or fat distribution with random genetic markers or candidate genes have been reported using the sibling-pair linkage method. One of the few reported studies relative to the visceral fat mass was the evaluation of a 122 sib-pair linkage analysis from the QFS between five microsatellite markers encompassing about 20 cM in the Mob-1 region of the human chromosome 16p12-p11.2 (the corresponding region in mice is significantly linked to body fat and blood lipids) (147). This study showed evidence of linkage between the marker D16S287, serum cholesterol and its fractions, and visceral fat ($P = 0.01$), while another marker (D16S401) located about 19 cM further centromeric also exhibited good evidence of linkage to abdominal visceral fat ($P = 0.007$). These results suggested to the authors that this region of the human genome contains a locus affecting the amount of visceral fat and lipid metabolism as also shown by the association studies indicated above. The other population and intrafamily association study used a polymorphic marker (LIPE) in the hormone-sensitive lipase gene, located on chromosome 19q13.1–13.2. This study suggests that the LIPE marker is in linkage disequilibrium with an allele and/or gene that increases susceptibility to abdominal obesity and thereby possibly to type 2 diabetes (148).

In conclusion, despite the fact that the genetic architecture

of obesity has just begun, the results obtained so far suggest that a great number of genes, loci, or chromosomal regions distributed on different chromosomes could play a role in determining body fat and fat distribution in humans. This reflects the complex and heterogeneous nature of obesity. The accumulation of adipose tissue in the abdominal region is at least partially influenced by genes, which becomes more evident as the number of involved genes are identified.

V. Adipose Tissue as an Endocrine Gland

The concept that adipocytes are secretory cells has emerged over the past few years. Adipocytes synthesize and release a variety of peptide and nonpeptide compounds; they also express other factors, in addition to their ability to store and mobilize triglycerides, retinoids, and cholesterol. These properties allow a cross-talk of adipose tissue with other organs as well as within the adipose tissue. The important finding that adipocytes secrete leptin as the product of the ob gene has established adipose tissue as an endocrine organ that communicates with the central nervous system.

A. Secreted proteins and triglyceride metabolism

1. LPL. As already mentioned, LPL is the key regulator of fat cell triglyceride deposition from circulating triglycerides. LPL is found, after transcytosis, associated with the glycosaminoglycans present in the luminal surface of the endothelial cells. The regulation of LPL secretion, stimulated by the most important hormonal regulator, insulin, is related to posttranslational changes in the LPL enzyme, at the level of the Golgi cisternae and exocytotic vesicles, insulin possibly having a positive role in this secretory process (149). Genes encoding LPL were not differentially expressed in omental when compared with subcutaneous adipocytes (150). However, in very obese individuals omental adipocytes express lower levels of LPL protein and mRNA than do subcutaneous fat cells (151). The regulation of LPL in obesity has been presented in the Section on correlations of abdominal visceral fat.

With respect to the hormonal regulation of LPL, insulin and glucocorticoids are the physiological stimulators of the LPL activity, and their association plays an important role in the regulation of body fat topography. In effect, omental adipose tissue is known to be less sensitive to insulin, both in the suppression of lipolysis (152) and in the stimulation of LPL (151). However, when exposed to the combination of insulin plus dexamethasone in culture for 7 days, large increases in adipose LPL were observed because of increases in LPL mRNA (151). Significant differences were observed between men and women. The omental/subcutaneous LPL mRNA ratio was higher in men than in women, and omental LPL was more responsive to insulin plus dexamethasone in men. The increase in LPL in response to dexamethasone suggests that the well known steroid-induced adipose redistribution (especially in the abdomen) may be caused by increases in LPL, which would lead to a preferential distribution of plasma triglyceride fatty acids to the abdominal depot. Therefore, these data suggest that LPL is central to the development of abdominal visceral obesity (98). On the other hand, catecholamines, GH, and testosterone (in males) reduce adipose tissue LPL (149).

2. Acylation-stimulating protein (ASP). ASP is considered the most potent stimulant of triglyceride synthesis in human adipocytes yet described. Its generation is as follows (99). Human adipocytes secrete three proteins of the alternate complement pathway: C3 (the third component of the complement), factor B, and factor D (adipsin), which interact extracellularly to produce a 77-amino-terminal fragment of C3 known as C3a. Excess carboxypeptidases in plasma rapidly cleave the terminal arginine from C3a to produce the 76-amino acid peptide known as C3a desarg or ASP, which then acts back upon the adipocyte, causing triglyceride synthesis to increase. As fatty acids are being liberated from triglyceride-rich lipoproteins and chylomicrons as the result of the action of LPL, ASP is also being generated and triglyceride synthesis increased concurrent with the need to do so. In human adipose tissue, in the postprandial period, ASP secretion and circulating triglycerides clearance are coordinated in accordance with the suggestion that ASP in sequence to LPL would have a paracrine autoregulatory role. The adipsin-ASP pathway, therefore, links events within the capillary space to the necessary metabolic response in the subendothelial space, thus avoiding the excess buildup of fatty acids in the capillary lumen. The generation of ASP is triggered by chylomicrons.

While insulin decreases gene expression of C3, B, and adipsin, it enhances the secretion of ASP as expected from the concurrent action of LPL and ASP. However, more intensely and independent of insulin, ASP is capable of stimulating triglyceride synthesis in adipocytes and fibroblasts. Thus, from the reduced sensitivity to insulin in the suppression of lipolysis and stimulation of LPL by the omental adipose tissue, omental obesity may represent an example of impaired activity of the ASP pathway (153) even if dysfunction of the pathway is a secondary feature. As a consequence, omental adipose tissue, as compared with subcutaneous fat tissue, would have a limited capacity to prevent fatty acids from reaching the liver, which may contribute to the abnormalities in metabolism observed in visceral obesity (153).

B. Secreted proteins and cholesterol and retinoid metabolism

1. Cholesteryl-ester transfer protein (CETP). Human adipose tissue is rich in CETP mRNA, probably one of the major sources of circulating CETP in humans. CETP promotes the exchange of cholesterol esters of triglycerides between plasma lipoproteins. Plasma CETP appears to be an important modulator of reverse cholesterol transport by facilitating the transfer of cholesterol esters from HDL to triglyceride-rich apoB-containing lipoproteins, particularly VLDL, which is converted to intermediate density lipoprotein, then low density lipoprotein (LDL), and is ultimately cleared by the liver via the apo B/E receptor system. In this way, the adipose tissue is a cholesterol storage organ in humans and animals; peripheral cholesterol is taken up by HDL species, which act as cholesterol efflux acceptors, and is returned to the liver for excretion (154, 155). The synthesis and/or se-

cretion of CETP in adipose tissue is increased by fasting, high cholesterol/saturated fat diet, and insulin stimulation.

The few studies of circulating CETP in obesity have shown that activity and protein mass of CETP are both significantly increased in obesity, being negatively correlated with HDL cholesterol and the cholesteryl ester-triglyceride ratio of HDL2 and HDL3, thus exhibiting an atherogenic lipoprotein profile. Furthermore, there was a positive correlation with fasting plasma insulin and blood glucose, suggesting a possible link to insulin resistance (156–158). From an observation of Angel and Shen (154), it could be suggested that the CETP activity of omental adipose tissue is greatly increased in comparison with subcutaneous fat.

2. Retinol-binding protein (RBP). Adipose tissue is importantly involved in retinoid storage and metabolism. RBP is synthesized and secreted by adipocytes (159), the rate of RBP gene transcription being induced by retinoic acid (160). The mRNA encoding RBP is expressed at a relatively high level in adipocytes with no difference between subcutaneous and omental fat cells (150). There are no data regarding retinol mobilization from adipose stores in humans; however, *in vitro* studies with murine adipocytes showed that the cAMP-stimulated retinol efflux from fat cells was not the result of increased RBP secretion but instead due to the hydrolysis of retinyl esters by the cAMP-dependent hormone-sensitive lipase (161).

C. Protein related to blood coagulation: plasminogen activator inhibitor-1 (PAI-1)

PAI-1 is a serine protease inhibitor and evidence suggests that it is a major regulator of the fibrinolytic system, the natural defense against thrombosis. It binds and rapidly inhibits both single- and two-chain tissue plasminogen activator (tPA) and urokinase plasminogen activator (uTPA), which modulate endogenous fibrinolysis. The major sources of PAI-1 synthesis are hepatocytes and endothelial cells, but platelets, smooth muscle cells, and adipocytes are also contributors (162). The increased gene expression and secretion of PAI-1 by adipose tissue contribute to its elevated plasma levels in obesity, presenting a strong correlation with parameters that define the insulin resistance syndrome, in particular with fasting plasma insulin and triglycerides, BMI, and visceral fat accumulation: omental adipose tissue explants produced significantly more PAI-1 antigen than did subcutaneous tissue from the same individual, and transforming growth factor-βl increased PAI-1 antigen production (163). In a premenopausal population of healthy women with a wide range of BMI, there was a positive correlation of PAI-1 activity with CT-measured visceral fat area, independent of insulin and triglyceride levels. The amount of visceral adipose tissue area explained 28% of the PAI-1 activity variance. Weight loss confirmed this link. PAI-1 diminution was correlated only with visceral adipose tissue area loss and not with total fat, insulin, or triglyceride decrease (164).

Results from *in vitro* studies have shown that insulin (165–168) stimulates PAI-1 production by cultured endothelial cells or hepatocytes. Attempts to extrapolate these *in vitro* data to *in vivo* proved difficult. Acute (2-h hyperinsulinemia) modulation of plasma insulin in humans did not affect PAI-1 levels, and hypertriglyceridemia from several origins was not always associated with increased PAI-1 levels (163). In the same way, exogenous (short-term) insulin infusion with triacylglycerol and glucose failed to demonstrate elevations of PAI-1 (169). The augmentation of PAI-1 by insulin probably requires concomitant elevation of lipids and glucose and perhaps other metabolites in blood, as suggested by the strikingly synergistic effects when Hep G2 cells are exposed to both insulin and fatty acids *in vitro* (170). Accordingly, a hyperglycemic hyperinsulinemic clamp associated with an intralipid infusion for 6 h, to induce hyperinsulinemia combined with hyperglycemia and hypertriglyceridemia, produced an increase in PAI-1 concentrations in blood for as long as 6 h after cessation of the infusion (171). However, the extent to which elevation of any one constituent or any given combination of elevations is sufficient to induce the phenomenon has not yet been elucidated in insulin-resistant patients. In effect, the reduction of PAI-1 after weight loss related more to the degree of weight reduction than to triglyceride or insulin changes, as above indicated, and the lack of increase of PAI-1 in type 2 diabetics without obesity (172), strongly suggesting that visceral fat is an important contributor to the elevated plasma PAI-1 level observed in visceral obesity independent of insulin, triglyceride, and glucose level. Finally, prospective cohort studies of patients with previous myocardial infarction (173) or angina pectoris (174) have underlined the association between an increase in plasma PAI-1 levels and corresponding defective fibrinolysis and the risk of atherosclerosis and thrombosis, particularly in relation to coronary events (162), thus linking visceral fat accumulation to macrovascular disease (163).

Recently, it was shown that in addition to insulin, corticosteroids (dexamethasone and hydroxycorticosterone) affect PAI-1 synthesis by human subcutaneous adipose tissue explants in a dose-dependent manner; this model showed the regulation of PAI-1 by adipose tissue after validation by showing a high correlation between the production of PAI-1 by omental and subcutaneous fat (175). In the same way, it was demonstrated that PAI-1 production was significantly correlated with that of tumor necrosis factor-α (TNFα), emphasizing a possible local contribution of TNFα in the regulation of PAI-1 production by human adipose tissue (176).

D. Secreted factors with an endocrine function

1. Estrogens. P450 aromatase activity in adipose tissue is important for estrogen production, which may have a paracrine role, since, as previously indicated, estrogen receptors are expressed in human adipose tissue (122). In effect, estrone, the second major human circulating estrogen in premenopausal women and the predominant one in postmenopausal women, is mostly derived from the metabolism of ovarian-secreted estradiol (catalyzed by 17β-hydroxy steroid dehydrogenase) and from aromatization of androstenedione in adipose tissue in the former and almost exclusively by aromatization of that C19 androgen secreted by the adrenals in the latter. The peripheral aromatization of testosterone to estradiol and estrone contributes minimally to estradiol and estrone production (177). The conversion rate of andro-

stenedione to estrone increases as a function of aging and obesity [due to an increase in adipose tissue P450 aromatase transcript levels, highest in the buttocks, next highest in the thighs, and lowest in the subcutaneous abdominal tissue (178, 179)] and significantly greater in women with lower (gynoid) obesity than in upper body obesity (180). In obese men, the peripheral conversions of testosterone to estradiol and androstenedione to estrone, as well as the circulating levels of those estrogens, are also increased in proportion to the degree of obesity (181, 182). However, only plasma levels of estrone had a significant correlation with CT-derived abdominal visceral fat and femoral areas (182). Since the increased metabolism of testosterone to estradiol did not account for the major increase in estradiol production in obese men (181), it is probable that estradiol is secreted or is produced from the peripheral conversion of estrone, as observed in postmenopausal women. The most abundant adrenal steroid, dehydroepiandrosterone sulfate (DHEA-S) can also form the active sex steroids, dihydrotestosterone and estradiol, in several tissues, including mesenteric fat (183). The active androgens and estrogens made locally in peripheral tissues, especially adipose fat, exert their action by interacting with the corresponding receptors in the same or nearby cells where their synthesis took place before being released in the extracellular environment as such or as inactive metabolites.

The aromatase enzyme responsible for transforming androstenedione into estrone is present in nonendocrine tissues, particularly adipocytes and adipose stromal cells, the level of aromatase activity in stromal cells being greater than that in adipocytes (184). It was shown that within abdominal subcutaneous stromal cells (preadipocytes), there are intrinsic gender differences in the regulation of aromatase by insulin + cortisol, which is specific for females. Mature adipocytes express aromatase, which is stimulated by insulin + cortisol in both sexes. Insulin and cortisol independently induce preadipocyte differentiation with both having a synergistic effect (185). The intrinsic gender differences in preadipocytes could contribute to a gender-specific pattern of fat distribution (182).

2. Leptin. Leptin is the product of the obesity (ob) gene, which is expressed in adipocytes (186, 187). The human ob gene spans approximately 20 kb and exists in a single copy on chromosome 7q32.1; it consists of 3 exons and 2 introns, with the leptin open reading frame formed from the 3′-end of exon 2 and the 5′-end of exon 3. The mouse ob gene is structurally similar to the human gene except for the presence of an additional untranslated exon between exons 1 and 2 that appears in approximately 5% of transcripts (188). The predicted amino acid sequence is 84% identical in human and mice leptin, having the features of a secreted protein (189). Several studies in rodents suggest that leptin acts as a signaling factor from adipose tissue to the central nervous system, regulating food intake and energy expenditure. It is hypothesized that via this leptin feedback loop, homeostasis of body weight and a constant amount of body fat are achieved (189). In humans, a strong positive correlation is observed between serum leptin levels and the amount of body fat and adipocyte leptin mRNA as in rodents (189, 190). The results are in accordance with the *in vitro* data indicating that leptin secretion is a reflection of fat hypertrophy. The adipocyte is the only known source of the ob gene product, leptin, as the preadipocytes do not present this capacity (188). By measuring levels of leptin mRNA by quantitative RT-PCR in adipocytes isolated from omental and subcutaneous adipose depots of nonobese and mildly obese individuals undergoing elective surgery, Montague and co-workers (191) have shown that leptin mRNA was greater in subcutaneous than in omental adipocytes ($P < 0.0001$). The subcutaneous-omental ratio of leptin mRNA expression was markedly higher in women than in men. Part of the results, according to the authors, could be explained, particularly in women, by the fact that subcutaneous adipocytes are larger than omental adipocytes and as adipocytes increase in size, the leptin mRNA is up-regulated such that it forms a greater proportion of the total mRNA than in smaller adipocytes. Indeed, increased leptin mRNA expression in large adipocytes has been reported by Hamilton *et al.* (192). Furthermore, leptin expression and levels increase as the size of the adipose tissue triglyceride stores increase (193). In a study examining the secretion of leptin in subcutaneous and omental fat tissue from obese and nonobese women, it was shown that the leptin secretion rate and leptin mRNA expression were about 2 to 3 times higher in the subcutaneous than in the omental fat tissue in both obese and nonobese subjects. There was a positive correlation between BMI and leptin secretion rates in subcutaneous and omental fat tissue. Furthermore, leptin secretion rates in both fat tissues had a high positive correlation with serum leptin levels. This study concluded that the subcutaneous fat depot is the major source of leptin in women owing to the combination of a mass effect, since subcutaneous adipose tissue is the major fat depot presenting a higher secretion rate due to enlarged cell size (subcutaneous adipocytes were ~50% larger than omental fat cells) and increased expression of the leptin gene (194). Serum leptin circulates, in part, bound to transport proteins in the serum of both rodents and humans, and the size distribution of endogenous serum leptin, as determined by RIA after sucrose gradient centrifugation, is consistent with saturation of binding in hyperleptinemic obesity. Thus, in humans, free leptin increases with BMI (195).

For individuals with the same BMI, the leptin circulating levels can vary by 1 order of magnitude (190), suggesting that leptin is regulated by factors other than the size of the adipose tissue depot. In effect, the secretion of leptin by adipocytes is regulated by nutritional and hormonal factors. Acute changes in energy balance appear to regulate leptin expression and circulating levels. In effect, an increase in caloric intake results in a sharp increase in serum leptin approximately 40% over baseline within 12 h, without changes in body weight (196). On the other hand, both leptin expression and levels decline rapidly in response to starvation, with serum leptin levels starting to decline after 12 h of fasting and reaching a nadir after 36 h, out of proportion to body adiposity changes (197, 198). Thus, under conditions of steady-state energy balance, leptin is a static index of the amount of triglyceride stored in adipose tissue and in non-steady-state energy balance situations. Leptin may be acutely regulated independently of the available adipose tissue triglyceride

stores and may serve as a sensor of energy balance (189). However, the precise mechanism mediating the distinct responses to changes in body adiposity and energy balance remains to be elucidated. In rodents, the decreased ob gene expression after fasting and increase after realimentation appear to be related, according to *in vitro* data, to a transcriptional direct effect of insulin (197, 199, 200). In humans, the positive effects of insulin are controversial *in vivo*. Although acute (≤3 h) insulin has no stimulatory effect, longer hyperinsulinemic clamps resulted in increased leptin concentrations in some, but not all, studies. Experiments *in vitro* have not solved the controversy over the potential effects of insulin on leptin synthesis, as both an increase and no change have been reported (201). Dose-response and time-course characteristics of the effect of insulin on plasma leptin in normal men during a 9-h euglycemic clamp indicated that physiological insulinemia acutely increases leptin by comparison with a control saline infusion. Plasma leptin also showed a dosage-dependent increase during the insulin infusion (202). The hormonal regulation (glucocorticoids and insulin) of leptin synthesis was studied by Halleux *et al.* (201) in cultured visceral adipose tissue from lean and obese patients, sampled during elective abdominal surgery. They found that glucocorticoids, at physiological concentrations, stimulated leptin secretion by enhancing the pretranslational machinery in human visceral fat. This effect was more pronounced in obese subjects due to a greater responsiveness of the ob gene. Unlike glucocorticoids, insulin had no direct stimulatory effect on ob gene expression and leptin secretion and even prevented the positive response to dexamethasone by a cAMP-independent mechanism that remained functional despite insulin resistance. Serum leptin concentrations in humans exhibit a sexual dimorphism, with circulating levels being higher in women than in men. Although women tend to have a higher fat mass than men for the same BMI, this dimorphism appears to occur independently of body adiposity (193). Two factors are related to the sexual dimorphism of serum leptin. The first is the higher ratio of subcutaneous to omental fat mass (7) and since a significantly higher subcutaneous-omental fat ratio of leptin expression was demonstrated in women, as above indicated (191), the higher serum leptin levels in women could reflect, at least partially, these gender variations in regional body fat distribution and leptin expression. The second factor is the prevailing sex steroid milieu. Cross-sex hormone administration in transsexual subjects showed that subjects with high circulating testosterone, whether male or female, had significantly lower serum leptin at a certain degree of body fatness compared with subjects (male or female) with high estrogen and low testosterone levels. These results indicated that sex hormone steroids, in particular testosterone, play an important role in the regulation of serum leptin levels, concluding that the prevailing sex steroid milieu, not genetic sex, is the significant determinant of the sex difference in serum lipids (203).

It was shown that TNFα positively modulates leptin secretion by adipocytes (204); thus, increased TNFα expression in adipose cells seen in obesity could be related to the hyperleptinemia found in this situation. In effect, a positive independent association was shown between circulating levels of leptin and of circulating soluble human 55-kDa TNFα receptor, which has been validated as a sensitive indicator of activation of the TNFα system (205) in healthy young controls and type 2 diabetics. This reflects an association between leptin and the TNFα system in humans similar to that seen in rodents, where TNFα and interleukins increase leptin gene expression and circulating leptin levels (204, 205).

All experimental studies indicated that the central nervous system is a major site of leptin action, inducing a reduction in activity of orexigenic and an activation of anorexigenic neurons (188, 206). Moreover, leptin may affect neuroendocrine mechanisms other than regulation of food intake, which will not be discussed in the present review. Furthermore, it is being increasingly appreciated that leptin may also act in the periphery. Thus, leptin has been shown to reduce lipid synthesis in cultured adipocytes as well as decrease triglyceride synthesis and increase fatty acid oxidation in normal pancreatic islet cells in short-term culture (207). Normal rats made chronically hyperleptinemic exhibit a prompt and sustained reduction in food intake and disappearance of all visible body fat, associated with hypoglycemia, as well as hypoinsulinemia associated with complete depletion of islet cell triglyceride content, unresponsive to *in vitro* stimulatory levels of glucose and arginine. This is restored by perifusion of the islets with an oleate/palmitate mixture. It was concluded that hyperleptinemia causes reversible β-cell dysfunction by depleting tissue lipids, thereby depriving β-cells of a lipid signal required for the insulin response to other fuels (208). This finding, in combination with the previous observation that insulin stimulates leptin secretion and the demonstration of leptin receptors on human islets β-cells, and that leptin suppresses insulin secretion and gene expression, suggests the existence of an adipoinsular axis in rodents and humans in which insulin stimulates leptin production in adipocytes, and leptin inhibits the production of insulin in β-cells (209). There are also actions of leptin on other organ systems, apart from the nervous system and endocrine-metabolic realms.

3. Angiotensinogen. Angiotensinogen is synthesized primarily by the liver and secreted abundantly by the adipose tissue. Its gene expression in fat tissue is regulated by glucocorticoids (210) and cleaved in the circulation by renin to angiotensin I, which is subsequently converted to angiotensin II by angiotensin-converting enzyme; both enzymes are also expressed in adipose tissue (211). Thus, angiotensin II, produced locally in adipose tissue, can induce preadipocytes to differentiate into adipocytes by stimulating prostacyclin production from adipocytes (149). It was found that in nonobese and obese rats, angiotensinogen protein and correspondent mRNA are about 2-fold higher in visceral adipose tissue than in subcutaneous sites, and its production increases concomitantly with the development of obesity in the obese Zucker rat (212, 213). Since adipose tissue constitutes the most important source of angiotensinogen after the liver, it cannot be excluded that, in addition to its effect on the development of adipose tissue, an enhanced secretion of angiotensinogen, via angiotensin II, could lead to the increased levels of blood pressure frequently observed in obesity (149). The angiotensinogen mRNA expressed in subcutaneous abdominal

adipocytes was greater in obese than in lean subjects, but not significantly so. Further, no significant differences were found between obese patients with and without hypertension in the small numbers of subjects studied (214).

4. Adiponectin. Through an extensive search of the human adipose tissue cDNA library, Matsuzawa and co-workers (215) isolated a novel cDNA encoding a collagen-like secretory protein that was named adiponectin. Adiponectin was demonstrated to be specifically and abundantly expressed in adipose tissue; it is detected in human plasma and analyzed in both by immunoblotting. In normal male subjects, plasma adiponectin levels were negatively correlated with BMI and visceral fat area but not with subcutaneous abdominal fat area. Plasma levels in patients with coronary heart disease were lower than those without heart disease, although no difference was observed in BMI or visceral fat area (216). To elucidate the regulation of plasma adiponectin in comparison with leptin levels, the same investigators studied rhesus monkeys with various body weights and also with and without type 2 diabetes. There was a significant inverse correlation between body weight and plasma adiponectin levels while, as expected, corresponding leptin levels correlated significantly with body weight. With respect to the insulin values, the plasma adiponectin decreased and leptin increased significantly in hyperinsulinemic monkeys. A longitudinal study in 13 monkeys revealed that the plasma adiponectin decreased as they gained weight, whereas the plasma leptin levels increased. It was concluded that the adiponectin levels would be negatively regulated by adiposity and that the plasma leptin levels were positively regulated by adiposity (217). It was shown that adiponectin inhibited growth factor-induced human aortic smooth muscle cell proliferation (215).

E. Factors with an autocrine/paracrine activity regulating adipose tissue cellularity

1. TNFα. Adipocytes are both a source of and a target tissue of the cytokine TNFα, which is absent in the preadipocyte although it is expressed in the adipocyte. Obese individuals express 2.5-fold more TNFα mRNA in fat tissue (subcutaneous abdominal fat studied) relative to lean controls (218, 219), with a significant correlation between TNFα mRNA and BMI. Similar increases were observed in adipose production of TNFα protein. In obese subjects, high circulating levels were reported, which fell significantly after weight loss (220). In addition, a strong positive correlation is observed between TNFα mRNA expression in fat tissue and the level of hyperinsulinemia, an indirect measure of insulin resistance. Regarding the molecular mechanism responsible for the decreased insulin action, especially in obesity, it appears to involve TNFα-induced serine phosphorylation of insulin-receptor-substrate-(IRS)-1 (221). Although there was heterogeneity in mRNA values among obese subjects, there was a consistent reduction in TNFα mRNA expression and protein level of approximately the same magnitude in adipose tissue after weight loss. In contrast to the marked site-related expression of leptin, as previously indicated, genes encoding TNFα are not differentially expressed in human subcutaneous and omental adipocytes (150). Since the expression of TNFα is negatively correlated with LPL activity in the adipose tissue and is higher in the reduced-obese subjects, the magnitude of these changes did not correlate with each other, suggesting that factors, other than adipocyte TNFα expression, are involved in regulating LPL in the reduced-obese state (218). Together, these studies could suggest a local action of the cytokine, in addition to the existence of some additional local factor, limiting the entrance of fatty acids via LPL and the subsequent hypertrophy of the adipocyte. In effect, in addition to the decrease in activity of LPL, TNFα has multiple actions in adipose tissue, including a decrease in expression of the glucose transporter GLUT 4 (222) and an increase in hormone-sensitive lipase (223). Therefore, the production of TNFα by adipose tissue could be a local regulator of fat cell size, and the overproduction of TNFα in adipocytes of obesity could represent a form of "adipostat," *i.e.*, a normal homeostatic mechanism designed to limit adipocyte size in the face of overconsumption (224). In a group of male patients with premature coronary heart disease, TNFα levels measured using a sensitive enzyme-linked immunosorbent assay (ELISA) for human TNFα did not show any relationships either with plasma insulin concentrations or the degree of insulin resistance as measured by the HOMA method (a crude measure of insulin resistance). It appeared from that study that the elevated TNFα circulating levels were associated with atherogenic metabolic disturbances in men with premature coronary heart disease (225). In line with this report is the observation that in subcutaneous adipose tissue taken from lean controls, obese insulin-resistant subjects with normal glucose tolerance, and obese insulin-resistant type 2 diabetics, all males, TNFα mRNA expression was normal in healthy obese men and type 2 diabetic patients; it was not regulated by hyperinsulinemia and was not associated with obesity or insulin resistance, as evaluated by an euglycemic hyperinsulinemic clamp (226). Accordingly, given the well established link between omental adiposity and insulin resistance in humans, if adipocyte TNFα expression is linked to insulin resistance (219), there should be evidence for a site-related TNFα expression in isolated human adipocytes that has not been observed (150). In addition, Montague *et al.* (150) found no correlation between TNFα and BMI in their subjects (BMI $< 35\ kg/m^2$). Analysis of the data presented by Kern *et al.* (218) indicated no significant relationship between BMI and TNFα expression in adipose tissue. However, if the morbidly obese subjects (BMI > 45) were excluded, such correlation becomes significant. In addition, preliminary data indicated a trend for a higher release of TNFα in omental than subcutaneous adipose tissue obtained from morbidly obese subjects (227).

2. Peroxisome proliferator activated receptor-γ (PPAR-γ). PPARs are ligand-activated transcription factors of the nuclear hormone receptor superfamily. Of the three distinct PPAR subtypes, PPAR-γ is highly tissue selective, being most abundant in adipose tissue. PPAR-γ exists in three isoforms, γ-1, -2, and -3. The nature of the endogenous ligand(s) to PPAR-γ is still unclear, although arachidonic acid metabolites such as 15-deoxy-δ-12,14-PGJ_2, and long-chain fatty acids have been implicated. Activation of PPAR-γ results in altered expres-

sion of selected target genes. PPARs, including PPAR-γ, are only transcriptionally active after heterodimerization with a 9-*cis* retinoic acid-activated receptor, retinoid X receptor (RXR). Selectivity of target gene response is conferred by binding of the PPAR-γ/RXR heterodimer to a specific DNA sequence in the 5′-flanking region. Such sequences have been identified in the regulatory regions of PPAR-γ-responsive genes [*e.g.*, GLUT 4, LPL, fatty acid-binding protein, carnitine palmitoyl transferase l (228, 229)].

In a comparison of the mRNA expression levels of PPAR-γ in subcutaneous and omental adipose tissue, Lefebve *et al.* (230) found significantly lower levels in visceral adipose tissue in subjects with a BMI < 30 kg/m^2 but not in obese subjects, indicating that relative PPAR-γ expression is increased in omental fat obesity. When the absolute PPAR-γ mRNA values were analyzed, there was no relation with BMI in subcutaneous adipose tissue. In the omental fat, however, a trend to a positive correlation was observed but it did not reach significance in the population tested, who exhibited a wide range of BMI. The same researchers found a 2-fold reduction in GLUT 4, glycogen synthase, and leptin mRNA expression in omental adipose tissue, suggesting a lower GLUT 4-mediated glucose uptake, and perhaps glucose storage, in omental adipocytes while the total insulin receptor expression was significantly higher in this tissue. Most of this increase was accounted for by expression of the differentially spliced insulin receptor lacking exon 11, which is thought to transmit the insulin signal less efficiently than the insulin receptor lacking exon 11. This finding is consistent with the reduction in GLUT 4 and glycogen synthase but partially at least for the decrease in leptin gene expression. This suggests that other regulators of that gene are more likely to participate in the depot-specific difference.

With respect to the expression of PPAR-γ splice variants, γ-1 and γ-2, it was demonstrated that PPAR-γ-l is the major isoform in human adipocytes by Western blotting (229). However, Vidal-Puig *et al.* (231), using an RNAse protection assay to examine the levels of the two isoforms in abdominal subcutaneous adipose tissue, reported no difference in gene expression of the splice variants in lean and morbidly obese individuals, but did demonstrate an increase in PPAR-γ-2 in the obese group (BMI > 43 kg/m^2). On the other hand, Auboeuf *et al.* (232) using a quantitative RT-competitive PCR showed that the PPAR-γ-l is predominant in adipose tissue while the γ-2 was a minor isoform with no difference in expression of PPAR-γ-1, in abdominal subcutaneous fat, between morbidly obese and lean subjects. In addition, the expression of PPAR-γ isoforms is modulated by caloric intake, *i.e.*, a low calorie diet specifically down-regulates the expression of PPAR-γ in adipose tissue of obese humans but increases it again during weight maintenance. The two adipogenic hormones, insulin and glucocorticoids, show a synergistic effect to induce PPAR-γ mRNA after *in vitro* exposition to isolated human adipocytes. *In vivo* modulation of human PPAR-γ mRNA by obesity and nutrition could suggest a possible role for PPAR-γ expression in the pathogenesis of altered adipocyte number and function in obesity (231). In effect, PPAR-γ has been shown to induce apoptosis of large adipocytes and the differentiation of small adipocytes *in vivo* (233). Because smaller adipocytes are usually more sensitive to insulin, such a differentiated response would be expected to produce greater insulin-dependent glucose uptake.

3. Interleukin-6. Increasing evidence points to the importance of locally produced cytokines in the regulation of adipocyte metabolism. Among the cytokines, in addition to TNFα, which increases with fat cell enlargement in obesity, adipose tissue also produces another ubiquitous cytokine, interleukin-6. Since the plasma concentration of interleukin-6 is proportional to the fat mass (234), the adipose tissue could become an important source of that cytokine. Since interleukin-6 as well as TNFα reduces the expression of LPL, it could have a local role in the regulation of the uptake of fatty acids by the adipose tissue. It is possible that adipose tissue TNFα, whose expression is increased in obesity, induces adipocyte and nonadipocyte interleukin-6 expression. In effect, TNFα produces a 60-fold increase in interleukin-6 production in differentiated 3T3-L1 adipocytes (235). It was demonstrated that fragments of omental adipose tissue release 2–3 times more interleukin-6 than subcutaneous abdominal adipose tissue, both obtained from severely obese subjects undergoing obesity surgery. Adipocytes isolated from the omental depot also secrete more interleukin-6 than those from the subcutaneous depot, but other cells within the adipose tissue made a greater contribution to the high release of that cytokine (235). Thus, interleukin-6 may be both an autocrine and a paracrine regulator of adipocyte function in addition to possible effects on other tissues, as stimulation of such acute phase protein synthesis and stimulation of the hypothalamic-pituitary-adrenal axis (236). Because the venous drainage from omental tissue flows directly into the liver, the metabolic impact of interleukin-6 release from omental adipose tissue may be of particular importance, since that cytokine increases hepatic triglyceride secretion (237), and may, therefore, contribute to the hypertriglyceridemia associated with visceral obesity.

It was demonstrated that cultures of adipose tissue from omental and subcutaneous adipose tissue with glucocorticoids down-regulate the production of interleukin-6. Since interleukin-6 directly stimulates adrenal cortisol release in addition to stimulating hypothalamic CRH and pituitary ACTH release (236), adipose tissue interleukin-6 may, therefore, act as a feedback regulator of hypothalamic-pituitary axis function. Cortisol suppression of adipose interleukin-6 production may serve as a feedback inhibitor of this regulatory loop (236), taking into consideration that increased cortisol turnover is a feature of visceral obesity, as will be discussed later in this review.

4. Insulin-like growth factor-1 (IGF-I). It was shown in preadipocytes from human subcutaneous fat tissue that adipose differentiation induced by the addition of cortisol, insulin, and l-T_3 to a serum-free culture medium was associated with an increase in IGF-I and IGF-binding protein 3 (IGFBP3) mRNAs, while the expression of IGF-I receptor (IGF-IR) mRNA remained relatively stable and the production of IGF-I and IGFBP3 increased greatly. In preadipocytes, human GH stimulated IGF-I and IGFBP3 mRNA expression as well as an increase in IGF-I and IGFBP3 production, possibly

increasing the disposal of free IGF-I. In differentiated adipocytes, human GH stimulated the expression and production of IGFBP3 but not of IGF-I, possibly decreasing the disposal of free IGF-I. The presence of cortisol led to a decrease of IGFBP3 expression and production in adipocytes. These data suggested an auto/paracrine action of IGF-I and IGFBP3 in human adipose tissue that can be modulated by GH and cortisol. In addition, it was shown that in human adipocytes IGF-1R is expressed in preadipocytes (238). In effect, it was shown in rodents that IGF-I mRNA expressed in preadipocytes, in the presence of GH and exogenous IGF-I, is then effectively translated and the resulting IGF-I, acting in an autocrine/paracrine fashion, induces the proliferation of the preadipocytes and their differentiation into adipocytes (149, 239).

5. Uncoupling proteins (UCPs). UCPs are mitochondrial membrane transporters that are involved in dissipating the proton electrochemical gradient, thereby releasing stored energy as heat. This implies a major role for UCPs in energy metabolism and thermogenesis, which are key risk factors for the development of obesity and other eating disorders. At present, three different UCPs have been identified by gene cloning: UCP-1 is expressed in brown adipocytes in rodents inducing heat production by uncoupling respiration from ATP synthesis; UCP-2 is widely expressed in human tissues; and UCP-3 expression is primarily limited to skeletal muscle, an important mediator of thermogenesis in humans (240). Using competitive RT-PCR as a measure, UCP-1 mRNA expression in the visceral adipose tissue of morbidly obese subjects was found to be at significantly lower levels in comparison to controls. In obese patients, UCP-1 mRNA levels exhibited a strong association with the UCP-1 promoter polymorphism, which was in complete association with four substitutions. Furthermore, there was a borderline significant association of UCP-1 mRNA abundance and the combined effect of Arg64Trp and Gln28Glu substitution of the β_3- and β_2-adrenergic receptor, respectively; the mutation of the β_3-adrenoreceptor was associated with lower lipolytic activity, suggesting that variant forms of adrenergic receptors implicated in obesity may affect UCP-1 expression (241). Kogure *et al.* (242) have also shown that there was an additive effect of one genetic variant of the UCP-1 gene (A to G change at −3,826 in the 5′-flanking domain of the gene) and the Trp64Arg mutation of the β_3-adrenergic receptor gene based on difficulty of weight loss in moderately obese Japanese women (242). However, genetic analysis of various human cohorts suggested a weak contribution of UCP-1 to control fat content and body weight (240). Oberkofler *et al.* have also demonstrated reduced UCP-2 mRNA expression levels in visceral adipose tissue in morbid obesity in comparison with control lean subjects. In both obese and nonobese individuals, UCP-2 mRNA abundance was higher in the intraperitoneal than in the extraperitoneal fat tissue, the UCP-2 mRNA expression not being significantly different between obese and nonobese subjects in the latter (243).

In conclusion, the reduction of UCP-1 and -2 mRNA in visceral adipose tissue associated with reduced gene expression of UCP-2, but not UCP-3, in skeletal muscle of human visceral obesity (244) is compatible with a decreased capacity to expend energy in subjects with visceral obesity. Although it is not yet proven that the level of UCP-1 and -2 mRNA expression predicts the amount of protein, the reduced expression of human UCP-1 and -2 gene in obesity opens the possibility of important mutations in these genes and/or gene coding for their regulatory proteins. The data indicating that UCP-2 and UCP-3 are involved in energy or proton conductance activities in humans are still quite weak, and the biochemical activities and biological roles of these newly described UCPs remain to be elucidated.

TABLE 4. Factors released or mainly expressed in the fat tissue

Factors released	Visceral fat	Subcutaneous fat
Lipoprotein lipase (LPL)	+	+
	+	++ (morbid obese)
Acylation stimulating protein (ASP)	+	++
Cholesteryl-ester transfer protein (CETP)	++	+
Retinol binding protein (RBP)	+	+
Plasminogen activator inhibitor-1 (PAI-1)	++	+
Estrogens	+	+
Leptin	+	++
Angiotensinogen	++	+
Adiponectin	++	+
Tumor necrosis factor-α (TNFα)	+	+
Interleukin-6	++	+
Insulin-like growth factor-I (IGF-I)	+	+
IGF-binding protein 3 (IGFBP3)	+	+
Monobutyrin	+	+
Factors expressed	**Visceral fat**	**Subcutaneous fat**
Peroxisomal proliferator activated receptor-γ (PPar-γ)	+	++
	++	+ (obese)
Uncoupling proteins (UCPs)		
UCP-1	+	++
UCP-2	+	++

4. Monobutyrin. In addition to the secreted ubiquitous angiogenic factors (TGFβ and PGE_2), monobutyrin (l-butyrylglycerol) is a specific secretion product of the adipocyte, favoring the vascularization of adipose tissue on development and vasodilation of the microvessels (245).

Table 4 summarizes the depot-specific differences in the expression and/or secretion of factors demonstrating the differences between visceral and subcutaneous fat tissue that can influence the development of regional variations in adipose tissue distribution.

VI. Body Fat Distribution and the Metabolic Profile

A. Comparison of lipolysis, antilipolysis, and lipogenesis in visceral abdominal and subcutaneous fat in nonobese and obese individuals

As clearly indicated earlier in this review, individuals with upper-body (central) obesity, *i.e*, fat accumulation in the subcutaneous abdominal and visceral depots, are prone to met-

abolic and cardiovascular complications, especially when there is excess fat in the visceral area. What is the mechanism behind regional fat distribution, and why is it more dangerous to accumulate fat in the visceral area than in other regions? The vascular anatomy and the metabolic activity of visceral fat may be the key factors predisposing to complications of obesity (36).

Only visceral adipose tissue is drained by the portal venous system and has a direct connection with the liver. Mobilization of FFAs is more rapid from visceral than from subcutaneous fat cells because of the higher lipolytic activity in visceral adipocytes, in both nonobese and obese individuals, particularly in the latter, which probably contributes significantly to the FFA levels in the systemic circulation (33). The higher lipolytic activity in visceral fat in comparison with the subcutaneous adipose tissue can be attributed, as indicated previously, to regional variation in the action of the major lipolysis-regulating hormones, catecholamines and insulin, the lipolytic effect of catecholamines being more pronounced and the antilipolytic effect of insulin being weaker in visceral than in subcutaneous adipose tissue (246). This site variation is related to the increased expression and function of β-adrenoreceptors (particularly β_3 associated with a decreased function of α_2-adrenoreceptor-dependent antilipolysis in the obese) and a decreased insulin receptor affinity and signal transduction in visceral adipocytes (Table 3). With respect to the antilipolytic effect of adenosine and prostaglandins produced in adipose tissue, it is equally or slightly more pronounced in subcutaneous than in visceral adipocytes (127) because of decreased agonist receptor number in visceral adipocytes (246). The visceral fat catecholamine-induced lipolysis is greater in obese men than in women; this is partially due to a larger fat cell volume and also to a greater β_3- and lower α_2-adrenoreceptor sensitivity (116), which results in higher FFA mobilization from visceral fat to the portal system in men than in women. On the other hand, the antilipolytic effect of insulin is reduced in omental adipocytes regardless of the presence of obesity (Table 3). Thus, the enhanced total lipolytic activity probably contributes significantly to the FFA levels in systemic circulation (32). However, in obesity, changes occur in adipocytes that conceivably try to offset the detrimental effects of accelerated lipolysis. For instance, although the lipolytic response to catecholamines is increased, the sensitivity of abdominal subcutaneous fat to catecholamine-induced lipolysis is decreased in obese women because of a depletion of β_2-adrenoreceptors (111). This adaptive mechanism of subcutaneous fat cannot be detected in visceral fat of obese individuals, in whom there are normal sensitivities of β_1 and β_2-adrenoreceptors but markedly increased sensitivity of β_3-adrenoreceptor-dependent lipolysis and severely decreased sensitivity to α_2-dependent antilipolysis (110) (Table 3).

With respect to the size of the fat depots with relation to LPL activity as well as acylation-stimulating protein (as indicated in the adipose tissue LPL activity in *Section IV*), it was demonstrated, as previously shown (21), that the uptake of triglycerides is higher in intraabdominal fat and, combined with rapid rate of release of glycerol, is a measurement of lipid mobilization. This is independent of the degree of BMI, and without correlation with LPL activity, which is expressed equally in human subcutaneous and omental adipocytes (191). In women, but not in men, the omental adipose tissue has smaller adipocytes, and it presents lower LPL activity than subcutaneous fat depots. The LPL activity is lower in visceral than in subcutaneous fat irrespective of the presence of obesity (Table 3). Thus, in visceral fat there is a higher turnover of lipids than in the other fat depots, with greater sensitivity to catecholamine-induced lipolysis and decreased sensitivity to insulin antilipolysis, featuring a depot tuned in a "lipolytic mode" (102). The opposite pattern corresponds to subcutaneous fat, which presents lower sensitivity to catecholamine-induced lipolysis and increased sensitivity to insulin, tuned in to a "liposynthetic mode" with low fractional lipid turnover. Obesity adds a generalized increase in lipid turnover sustained by an increased response to lipolytic agents, a reduced effect of antilipolytic hormones, and increased LPL activity, which is most likely due to chronic hyperinsulinemia and playing a role in maintaining excess body fat depots (Table 3).

B. Abdominal visceral fat and insulin sensitivity: role of FFAs

Since intraabdominal visceral fat has the highest fractional lipolytic rate in comparison with the other depots, it plays a quantitative role in whole-body lipolysis (~26% of upper body release of FFAs) that goes far beyond its absolute mass; by virtue of its anatomy it is also able to exert its influence on liver metabolism (247). Thus, the visceral fat mass probably contributes significantly to the FFA levels in the systemic circulation (33). However, the elevated exposure of the liver to FFAs from visceral fat in obesity was deduced indirectly rather than measured directly. Available information does not indicate that visceral adipose tissue contributes much to liver exposure of FFA (248). There is, however, a possibility that, by the addition of portal FFA and FFA in the hepatic artery, the liver is exposed to more than would be predicted from systemic FFA availability data (115, 249).

In effect, when the metabolic parameters in 50 obese women were compared, no significant differences were observed in the postabsorptive levels of FFAs between those presenting with an abdominal visceral area, measured by CT at the umbilicus, ≥ 65 cm^2 or lower than 65 cm^2 (250). Similarly, excess visceral adipose tissue accumulation in 43 men (CT-determined abdominal adipose visceral area at L4-L5 level ≥ 130 cm^2) with a wide range of BMI was associated with normal fasting FFA, but elevated FFA levels in the postprandial state were associated with an impaired clearance of plasma triglycerides (251).

The elevated FFA flux into the liver would decrease the hepatic insulin extraction by inhibiting insulin binding and degradation (252), leading to systemic hyperinsulinemia as well as inhibiting the suppression of hepatic glucose production by insulin (253, 254). In addition, FFAs accelerate gluconeogenesis by providing a continuous source of energy (ATP) and substrate (255). Finally, in response to the increase in FFA availability, an increased esterification of FFAs and reduced hepatic degradation of apolipoprotein B lead to an increased synthesis and secretion of small VLDL particles (251) (Fig. 3) with a decreased ratio of VLDL triglyceride to

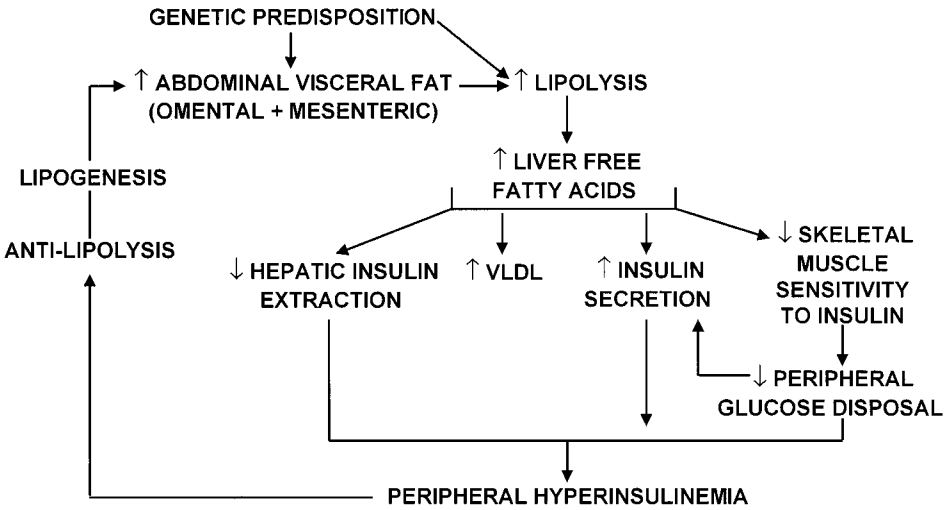

FIG. 3. Factors regulating the abdominal fat distribution. [Adapted with permission from B. L. Wajchenberg *et al.*: *Diabetes Metab Rev* 10:19–29, 1994 (256).]

apoB compared with the normal state. Furthermore, central obesity with insulin resistance and increased FFA levels is associated with increased hepatic lipase activity, which leads to removal of lipids from LDL and HDL, making them smaller and more dense. Thus, hepatic lipase activity is a major determinant of LDL size and density and the amount of HDL 2 cholesterol (251).

In addition to the effects on the liver, the increase in FFA flux induces a decrease in insulin-stimulated peripheral (primarily in skeletal muscle, the most relevant site of insulin resistance) glucose disposal, which in normal subjects is compensated by FFA-induced potentiation of glucose-stimulated insulin secretion (255). With the associated reduction in hepatic insulin extraction, this would determine still greater peripheral hyperinsulinemia (Fig. 3). With hyperinsulinemia, which nevertheless inhibits lipolysis of insulin-sensitive subcutaneous adipocytes, the fraction of systemic FFAs originating in visceral fat may be further expanded (33). In obese individuals who are genetically predisposed to develop type 2 diabetes, FFAs may eventually fail to stimulate insulin secretion sufficiently, leaving hepatic and peripheral insulin resistance unchecked and resulting in hepatic glucose overproduction and peripheral underutilization of glucose.

In contrast to the originally postulated mechanism in which FFAs were thought to inhibit insulin-stimulated glucose uptake in muscle through initial inhibition of pyruvate dehydrogenase (257), more recent studies have demonstrated that FFAs induce insulin resistance in obesity and type 2 diabetes by initial inhibition of glucose transport. Individuals with diabetes also have a decreased rate constant for glucose phosphorylation, which is then followed by an approximately 50% reduction in both the rate of muscle glycogen synthesis and glucose oxidation (258, 259).

In relation to the hyperinsulinemia of obesity, as reviewed by Boden (255), numerous studies have demonstrated that elevated release of FFA from adipose tissue inhibits insulin-stimulated glucose utilization, as indicated above. In addition, the accumulation of triglyceride in muscle has been linked to impaired glucose disposal (260). Also, the failure to normally suppress postprandial FFA availability in individuals with upper body obesity (despite a significantly greater postprandial insulinemic response than lower body obese and nonobese subjects) might impair the ability of insulin to suppress hepatic glucose output and stimulate glucose uptake in this particular phenotype (251). Since insulin secretion in obese subjects appears to be particularly sensitive to circulating FFA levels, it is attractive to suppose that increased availability of FFA directly stimulates the pancreatic β-cell while concomitantly contributing to insulin resistance in such individuals. As suggested by Unger (261), since FFA-induced changes in tissues (increase in fatty acid acyl-CoA) are proportional to the levels of FFA, the insulin resistance and insulin hypersecretion are matched and glucose tolerance is normal. If FFAs subserve this dual role (in addition to their other cellular functions), it would provide a simple mechanism whereby at any given time, the β-cell can "sense" how much insulin the muscle bed needs to maintain euglycemia in the early stages of obesity/type 2 diabetes syndrome. Consistent with this notion would be the correlation seen between pancreatic triglyceride content and glucose-stimulated insulin secretion in rat models with varying degrees of adiposity (262). In effect, when obese subjects become diabetic, FFAs rise to still higher levels with proportionally higher tissue levels of FACoA (fatty acid acyl CoA) being reflected by greater accumulation of triglycerides. In muscle, this intensifies insulin resistance, while islets, which respond fully to moderate FFA overload, are incapable of further increasing insulin secretion to match the peripheral insulin resistance. The greatly increased islet FACoA impairs the ability of the β-cells to respond to postprandial hyperglycemia, the hyperinsulinemia no longer matches the increase in insulin resistance, and thus type 2 diabetes begins (261).

We have demonstrated that the overnight lowering of plasma FFA levels with the potent, long acting nicotinic acid analog acipimox could improve insulin resistance in obese subjects exhibiting a wide spectrum of insulin sensitivities ranging from normal to diabetic (263). The decrease in basal

plasma FFA by an average of approximately 60% was associated with a reduction of approximately 50% of basal insulin levels and a decrease in basal glucose lower in the nondiabetics (~7%) but higher in the diabetics (~15%). This suggests that basal plasma FFAs exert a physiological important effect supporting up to one half of basal insulin levels in nondiabetic and diabetic subjects and that basal plasma FFAs are responsible for some of the hyperinsulinemia in normoglycemic obese subjects (264). The decrease in FFA was associated with an increase in insulin-stimulated glucose uptake (ISGU) during an euglycemic hyperinsulinemic clamp. The increase in glucose infusion rate (GIR), reflecting the ISGU, was relatively small in (23 ± 4%) in the lean nondiabetic controls, in whom acipimox produced only a modest decrease in plasma FFA. In contrast, in the obese nondiabetic with impaired glucose tolerance and in mild type 2 diabetes, the greater reduction in FFA was associated with an increase in GIR more than approximately 2-fold. The 131% increase in GIR was sufficient to normalize insulin sensitivity in the obese nondiabetic subjects, suggesting that elevated plasma FFA levels had been responsible for most of their insulin resistance. On the other hand, in obese subjects with impaired glucose tolerance or diabetes, doubling of insulin-stimulated glucose uptake was not sufficient to normalize their insulin sensitivity, which remained approximately 50% below that of lean nondiabetic controls. This suggested that elevated FFAs were responsible for about one half of their insulin resistance and confirmed previous findings by Boden and Chen (265) showing that plasma FFA levels could account for maximally 50% of insulin resistance in type 2 diabetes (Fig. 4).

All studies clearly suggest that in humans, as in rodents, glucose-fatty acid cross-talk within the β-cell is critically important for control of insulin secretion. In addition, obesity, a condition associated with more prolonged endogenous hyperlipacidemia, has been shown to enhance β-cell dependence on circulating FFAs, resulting in basal hyperinsulinemia and increased responsiveness to a glucose load. These findings complement other studies revealing that chronic exposure to very high levels of exogenous FFAs increases basal insulin levels and impairs glucose-stimulated insulin secretion. The developing picture is that endogenous FFAs enhance insulin secretion in obesity/insulin resistance syndromes, but eventually accumulate to toxic levels and contribute to β-cell failure and the development of type 2 diabetes (266). However, it should be emphasized that whether chronic hyperlipacidemia can actually cause impairment of β-cell function in both rodents and humans is still a matter of debate.

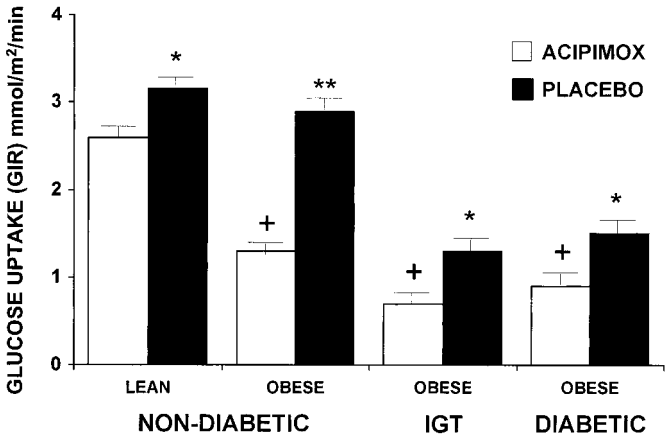

FIG. 4. Rates of glucose infusion needed to maintain euglycemia during hyperinsulinemic clamping (GIR) in lean and obese nondiabetic subjects and in subjects with IGT and type 2 diabetes after overnight treatment with placebo (*open bars*) or acipimox (*black bars*). Statistical analysis: *, $P < 0.001$; **, $P < 0.0001$ comparing placebo *vs.* acipimox treatment; +, $P < 0.001$ compared to lean nondiabetic controls. [Reprinted with permission of A. T. M. G. Santomauro *et al.*: *Diabetes* 48:1836–1841, 1999 (263).]

C. Glucose, insulin, lipid, and protein metabolism and its relationship to fat topography

Several studies have demonstrated that in obesity, the regional distribution of adipose tissue is correlated strongly with a number of important metabolic variables, including plasma glucose, insulin, and lipid concentrations (increased plasma total cholesterol and triglyceride and decreased plasma HDL cholesterol concentrations), as indicated in the *Introduction* (17–24). In effect, in obese, but not lean, men and premenopausal women the adipose tissue area measured by CT was positively correlated with fasting plasma glucose and insulin and C-peptide levels and with glucose and insulin areas under the curve after a 75-g glucose tolerance test. In addition, it was shown that the effect of accumulation of deep abdominal fat on glucose tolerance was independent of total adiposity. On the other hand, while the subcutaneous abdominal adipose tissue area correlated significantly with the glucose area under the curve in lean and obese men but not independently from the percentage of body fat, in obese females the subcutaneous abdominal fat was not significantly correlated with the glucose area (18, 19). These findings, as indicated before, suggested that subjects with visceral abdominal obesity are more insulin resistant than subjects with peripheral or lower body obesity. Indeed, Kissebah and Peiris (249) indicated that in obese premenopausal women, a central pattern of fat distribution (high WHR) is associated with a greater degree of insulin resistance. Similarly, an inverse relationship between central fat content and insulin-mediated glucose disposal was found by Lillioja *et al.* (267) in a mixed group of obese and nonobese males. The same findings were presented by Park *et al.* (20) and Märin *et al.* (21), who evaluated the intraabdominal fat mass by CT, rather than by using anthropometric measurements, in a group of nonobese and obese men. However, little information is available about the relationship between body fat distribution and *in vivo* insulin sensitivity in nonobese subjects. In effect, Landin *et al.* (112) found that fat distribution evaluated by WHR was poorly associated with insulin-mediated glucose disposal in nonobese females. Similarly, Bonora *et al.* (268) observed that in nonobese women no relationship was found between glucose metabolism in the basal state and during an euglycemic hyperinsulinemic clamp combined with indirect calorimetry (determining total, oxidative, and nonoxidative glucose disposal) and WHR, visceral and subcutaneous abdominal fat areas, measured by MRI, and visceral/subcutaneous fat area ratio, respectively.

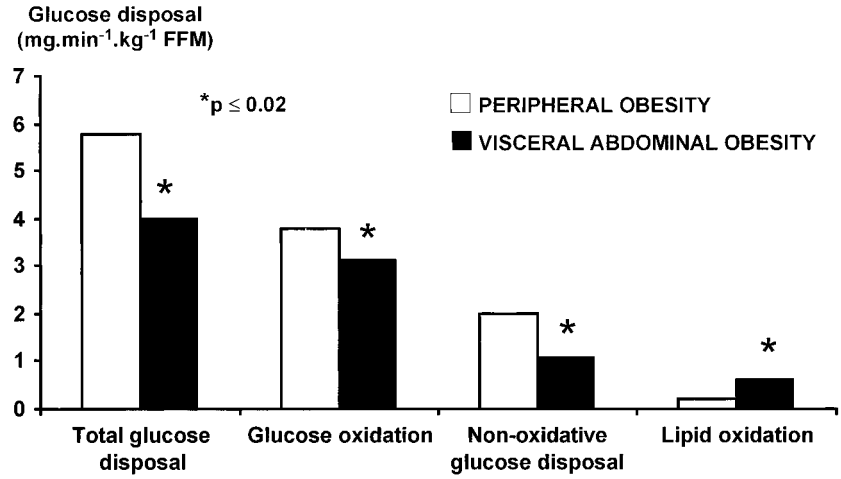

FIG. 5. Mean rates of total, oxidative and nonoxidative glucose disposal and lipid oxidation during an euglycemic clamp in obese women with peripheral obesity *vs*. visceral abdominal obesity (after controlling for the independent effect of total body fat content). [Derived from Ref. 269.]

According to these authors (268), such findings might be explained by the fact that in these subjects an insufficient amount of visceral fat had accumulated to exert a major impact on whole body glucose metabolism. The subject of visceral adipose fat in individuals of normal BMI will be discussed in *Section VII*.

Bonora (269) and Solini *et al.* (270) examined the relationship between obesity phenotypes and glucose, lipid, and protein metabolism in the basal state and during insulin infusion (euglycemic hyperinsulinemic clamp associated with indirect calorimetry) in obese women who had an assessment of subcutaneous and visceral abdominal fat by MRI and total body fat by tritiated water dilution. These authors observed that the subjects with visceral abdominal obesity, when compared with those with predominantly peripheral obesity, had significantly lower total glucose disposal, glucose oxidation, and nonoxidative glucose disposal and significantly greater lipid oxidation (Fig. 5) and reduction in total, oxidative, and nonoxidative leucine disposal during insulin infusion. Protein oxidation was positively related to glucose oxidation and negatively related to lipid oxidation. However, the rates of protein metabolism in the basal state and in the insulin concentrations encountered after a meal were normal, indicating that protein metabolism in obese patients does not differ appreciably from nonobese nondiabetic individuals. A major finding of this study (270) was that visceral fat was inversely related to endogenous leucine flux (an index of proteolysis) during hyperinsulinemia, indicating that visceral fat in obese women was associated with a greater sensitivity to the antiproteolytic effect of insulin, probably resulting from interactions between insulin and other glucoregulatory hormones, particularly anabolic steroid hormones; alternatively, higher FFA levels and the increased rate of lipid oxidation, characteristic of individuals with visceral obesity, may exert a protein-sparing effect (270).

In these women, as expected, visceral fat area was positively correlated to plasma FFA levels and the rate of lipid oxidation and inversely correlated to total glucose disposal, glucose oxidation, and nonoxidative glucose disposal during insulin infusion. All these relationships were independent of total and subcutaneous fat. In a group including both nondiabetic and diabetic subjects, it was also found that the amount of visceral fat was positively and independently correlated to endogenous glucose production during insulin infusion. As a whole, these results again suggested that an excess of visceral fat results in detrimental effects on glucose metabolism, the excess of FFA being the link between central fat and insulin resistance; however, other molecules released by visceral fat as well as other endocrine, metabolic, and hemodynamic abnormalities associated with visceral obesity might play a significant role. Furthermore, these studies also indicate that excess visceral adiposity also affects protein metabolism (270).

To determine the influence of sex in the relationship of visceral adipose tissue and glucose disposal, Banerji *et al.* (271) studied black men and women with type 2 diabetes, with a normal or slightly increased BMI, measuring insulin-mediated glucose disposal by the euglycemic clamp, and measuring total fat, as well as subcutaneous and visceral abdominal fat, by CT. Despite body composition differences, an inverse nonlinear relationship was observed between glucose disposal and visceral fat independent of sex; the slope and intercept were not different in men and women. Visceral fat explained a significant portion (34%) of variance in insulin-mediated glucose disposal, whereas total or subcutaneous fat did not.

To assess insulin sensitivity (SI sensitivity index, which measures the ability of insulin to enhance glucose disappearance and inhibit hepatic glucose production) and first and second phase β-cell sensitivity from plasma glucose, insulin, and C-peptide concentrations during a frequently sampled intravenous glucose tolerance test, the minimal model method was used (272, 273) in a group of normal weight and obese subjects with normal glucose tolerance test. It was shown that in the obese individuals of both sexes, as expected, insulin sensitivity was impaired and the β-cell hyperresponse to glucose was mainly due to enhanced second phase β-cell secretion. There was a negative correlation

of SI with BMI and visceral abdominal adipose fat area by CT. While the degree of visceral fat deposition affected insulin secretion, as there was a positive correlation between the amount of visceral adipose tissue and second phase insulin secretion as well as worsened insulin sensitivity, it did not influence energy expenditure evaluated by the resting metabolic rate adjusted for fat-free mass and glucose-induced thermogenesis in comparison with the lean controls (274).

With respect to the hepatic and peripheral insulin sensitivity in obesity, it was demonstrated, by using the euglycemic clamp technique, that in upper body obese women the liver is less sensitive but normally responsive to insulin, whereas peripheral tissues are both less sensitive and less responsive to insulin. While the dose-response curve for glucose utilization in lower body obese individuals is shifted slightly to the right and maximal glucose utilization is normal, the upper body obese curve exhibits a greater rightward shift and a marked reduction in maximal glucose utilization, suggesting that both insulin sensitivity and responsiveness are reduced, and that the defect in glucose utilization involves a rate-limiting step.

Compared with nonobese subjects, obese individuals have greater pancreatic insulin production, as is well known. It was shown (275) that in age- and weight-matched upper and lower body obese subjects evaluated by the WHR, pancreatic or prehepatic insulin production, estimated by the measurement of peripheral C-peptide turnover, was significantly correlated with the degree of overweight but not with WHR, suggesting that insulin hypersecretion is primarily related to total body fat mass but uninfluenced by the distribution of body fat or the degree of insulin resistance. However, the upper body or visceral obese have a decrease in hepatic insulin extraction, as previously indicated (24) (Fig. 3), basally and during intravenous or oral glucose challenge (249). Thus, within the obese group, increasing WHR was shown to be correlated with decreasing hepatic insulin extraction. Consequently, posthepatic insulin delivery was progressively increased and correlated well with the degree of peripheral hyperinsulinemia. Furthermore, the diminished hepatic insulin extraction in upper body obese subjects is proportionate to the magnitude of decline of peripheral insulin sensitivity.

Finally, the insulin metabolic clearance, determined during insulin infusion rates of 10, 20, 40, and 300 mU/min/m^2, during the euglycemic clamp, demonstrated that women with upper body obesity had a significant decline at submaximal and supramaximal insulin concentrations, suggesting that both receptor and postreceptor mechanisms are involved.

All data suggested that 1) the increased insulin production in abdominal obesity cannot completely compensate for insulin insensitivity; 2) the ability of the insulin-secretory process to sense the marked increase in peripheral insulin demand may be limited or defective; and 3) that a decline in insulin clearance is necessary to produce the systemic hyperinsulinemia, increasing WHR being associated with decreasing insulin metabolic clearance (249). As previously mentioned, an important factor for the hyperinsulinemia of visceral obesity might be the increased levels of FFA flux to the liver; Hennes *et al.* (276) examined the relative contributions from FFA and glucose to hepatic insulin extraction and peripheral hyperinsulinemia. Maintaining a high FFA flux while raising plasma glucose levels significantly increased plasma insulin concentrations, as a result of both increased pancreatic insulin secretion and decreased insulin clearance. The suppressive effects of glucose and FFA on endogenous insulin clearance were independent and additive. Thus, this study (276) confirmed that one potential mechanism explaining the hyperinsulinemia in abdominal visceral obesity might be the increased levels of FFA, particularly during hyperglycemia. The reduction of hepatic insulin uptake in abdominal visceral obesity may also be associated with an increase in androgenic activity (36).

VII. Pathology of the Abdominal Visceral Fat Without a Specific Endocrine Disorder

A. Increase in abdominal visceral fat

1. Normal BMI. Nearly 20 yr ago, Ruderman *et al.* (277) indicated that some individuals who are not obese on the basis of height and weight, like people with overt obesity, are hyperinsulinemic, insulin-resistant, and predisposed to type 2 diabetes, hypertriglyceridemia, and premature coronary heart disease, are now referred to as presenting insulin resistance or metabolic syndrome or syndrome X (256, 278, 279). Direct support for the concept of "metabolically obese" normal weight individuals [the dividing line between normal weight and overweight individuals being considered a BMI of 27 by Ruderman *et al.* (280)] who are prevalent in the general population was provided by the observation that during an euglycemic hyperinsulinemic clamp in 100 normal volunteers, nonobese or slightly obese with a BMI below 30 kg/m^2, there were individuals in the lowest quartile for glucose disappearance, 2.5 times lower than in the quartile 4, presenting the same BMI values, and having the highest insulin response during the oral glucose tolerance test, clearly establishing that insulin resistance and hyperinsulinemia occur together in a relatively nonobese, normoglycemic population (281). Subsequently, Zavaroni *et al.* (282) reported modestly higher systolic and diastolic blood pressure and plasma triglycerides and lower HDL cholesterol levels in nonobese male Italian factory workers with basal hyperinsulinemia than in normoinsulinemic men matched for age and weight. In addition, they noted that the hyperinsulinemic individuals had significantly higher plasma glucose levels after an oral glucose challenge that were still within the accepted normal range. Thus, this study confirmed the finding of Hollenberg and Reaven (281) that hyperinsulinemia and insulin resistance are common in nonobese Caucasian males and established that multiple risk factors for coronary heart disease are more prevalent in such individuals. Finally, the prevalence of diseases associated with insulin resistance (diabetes and coronary heart disease) increases as BMIs increase from 20 to 27 kg/m^2, with still further increases at higher BMIs (280).

Among the possible contributing factors often associated with pathogenesis of hyperinsulinemia and insulin resistance in both normal-weight ("metabolically obese") and obese individuals are visceral obesity, low birth weight, inactivity, and family history. The major points follow:

1. Visceral obesity. Most "metabolically obese" normal-

weight subjects have some increase in adipose tissue mass and insulin resistance probably due to an increase in visceral fat (280).

2. Visceral obesity, like hyperinsulinemia and insulin resistance, not only accompanies but antedates the components of the metabolic syndrome (8, 13, 283, 284). The correlation between BMI and visceral obesity can vary considerably from one individual to another. Thus, subjects with a relatively low BMI, such as "metabolically obese" normal-weight individuals, can have gross increases in abdominal visceral fat (20, 283), and others with a high BMI may have very little intraabdominal (visceral fat) (95).

3. Visceral obesity is the initial event that leads to insulin resistance by causing FFA levels to increase in the liver. This subject has been discussed previously. It should be mentioned that no association was observed between the visceral adipose tissue area and insulin-glucose homeostasis in nonobese men (19) and women (268). This is probably due to the fact that in these subjects, an insufficient amount of visceral fat had accumulated to attain a threshold value for deterioration of the metabolic profile (Table 2). However, visceral obesity will interact with genetic susceptibility in modulating the risk of metabolic complications associated with a given excess of visceral adipose tissue, as indicated in the *Introduction* (28). In addition, ethnic differences must be taken into account (285), particularly in those who have adopted a Western lifestyle.

4. Aging is associated with an increase in abdominal visceral fat as evaluated by waist girth, independent of changes in total adiposity. Waist circumference accounts for more than 40% of the variance in insulin sensitivity (euglycemic hyperinsulinemic clamp), whereas age explained only 10–20% of the total variance and less than 2% of the variance when the effects of waist girth were statistically controlled (286).

5. Low birth weight and low weight at l year of age have been linked to the metabolic syndrome in middle-aged and elderly individuals (287, 288) and include many classified as "metabolically obese" normal weight individuals. A low birth weight predisposes to insulin resistance and associated disorders independently of attained BMI in adult life (288, 289). Thus, many low-birth weight subjects have BMIs less than 24–26 kg/m^2 when middle-aged and would be classified as "metabolically obese" normal-weight individuals. Some data suggest that low-birth weight babies have central adiposity in middle age (287, 289) although definitive measurements of visceral fat are still lacking. Whatever the mechanism, the close relationship between birth weight and subsequent development of insulin resistance and associated disorders suggests that intrauterine nutrition could be a factor in causing susceptible individuals to eventually develop these problems. The fact that the majority of individuals with insulin resistance in midlife were not low-birth weight infants, however, indicates that it is not the sole factor (see Ref 280).

6. Inactivity and decreased fitness (low $VO_{2\ max}$). It has been shown that decreased fitness (low $VO_{2\ max}$) is associated with increased intraabdominal adiposity in offspring of patients with type 2 diabetes (290) and in a variety of patient groups (291) and is strongly correlated with an impaired insulin sensitivity (280).

The low $VO_{2\ max}$ cannot be explained by inactivity alone, because differences in aerobic fitness persist when insulin-resistant patients with type 2 diabetes and healthy offspring of parents with type 2 diabetes and control subjects with apparently similar lifestyles are compared (292, 294). A strong genetic contribution to the $VO_{2\ max}$ has been reported (295), raising the possibility that the low fitness level in patients with insulin resistance, including the "metabolically obese" normal weight individuals is, in part, genetically determined and may be an earlier indicator of insulin resistance than obesity *per se*. The finding of a decreased $VO_{2\ max}$ in young patients before the development of disorders associated with insulin resistance syndrome, even in normal-weight individuals, raises the possibility that decreased fitness and/or physical activity are important factors in their development. Since, as previously indicated, the genetic effect was 56% for the visceral fat area after adjustment for age, sex, and total fat mass in the Quebec Family Study (130), it is possible that central adiposity, low birth weight, and inactivity, in concert and independently, contribute to the development of insulin resistance in both normal-weight and obese individuals. Patients with type 2 diabetes, their insulin-resistant relatives, and subjects with visceral obesity with and without diabetes have been demonstrated to have a lower capillary density and a greater white-to-red muscle fiber ratio than do comparable control subjects. To what extent this reflects genetic differences and to what extent it reflects differences in physical activity status, however, remains to be determined (280).

7. Physical activity. Whatever the etiology of the low level of aerobic fitness in patients with insulin resistance, its presence strongly suggests a therapeutic role for physical activity. A considerable body of evidence indicates that glucose tolerance and insulin sensitivity, lipid parameters, blood pressure, and fibrinolytic activity can be improved by regular exercise in insulin-resistant individuals (296). The exercise need not be intense, considering that long-term walking (297) and jogging (298) programs that produce no or little change in $VO_{2\ max}$ have been shown to produce significant improvements in insulin resistance, plasma lipids, and blood pressure. However, in long-term studies, changes in adiposity make it difficult to isolate the effects of physical activity *per se*. It was shown that endurance training (5 days/week, for 6 months) in healthy elderly subjects, when compared with healthy young male individuals, with the same BMI and lean body mass, induced in both a significant increase in $VO_{2\ max}$, but the percentage increase was greater in the elderly (who had a significantly lower basal $VO_{2\ max}$). With respect to the changes in body composition, the most significant was a greater reduction in visceral abdominal fat area (at least 20%) in the older subjects, who at the start of the exercise presented twice the area in comparison with the young controls. With respect to the plasma lipids, there was a significant reduction in triglycerides in the older but not in the young subjects and an increment in HDL cholesterol in both elderly and younger individuals (J. Schwartz, personal communication). A study done in young and middle aged women, both trained and sedentary, indicated that the percent of body fat, as well as

the subcutaneous fat mass, was lower in trained than in sedentary individuals; both in the young and middle-aged subjects, the visceral fat mass remained lower than in sedentary individuals (299). From all studies, it can be concluded that improvements produced by regular exercise are greatest in individuals with central obesity and manifestations of the insulin resistance syndrome.

In a review of the effects of diet- and exercise-induced weight loss on visceral adipose tissue distribution in both men and women, Ross (300) mentioned that a diet-induced loss of approximately 12 kg corresponds to a 30–35% reduction in visceral fat mass. Regarding the effects of exercise *per se* on visceral fat, there appears to be a relative resistance to visceral adipose tissue reduction in obese women, whereas as previously mentioned (32), exercise-induced weight loss is associated with significant reductions in visceral fat in men. It was also reported that in obese men, reductions in visceral fat induced by the combination of diet and exercise are not different from those observed in response to diet alone (32). It is unclear whether the results of these studies reflect a biological truth or are confounded by methodological problems associated with the control of energy intake and expenditure in free-living patients.

In conclusion, it can be postulated that the improvement in insulin sensitivity by regular exercise in individuals with visceral obesity and insulin resistance is associated with a disproportionate loss of visceral fat. The finding of a decreased $VO_{2\ max}$ in young inactive patients before the development of disorders associated with insulin resistance syndrome suggests that decreased fitness and/or physical activity are important factors in their development. It is possible that visceral obesity, which often accompanies decreased fitness, contributes to the association between inactivity and insulin resistance. Since regular exercise ameliorates the entire cluster of metabolic and homeostatic abnormalities found in patients with insulin resistance and in addition tends to reverse the abnormal body composition and fat distribution found in these individuals, it can be an indication that the apparent effectiveness of regular exercise in decreasing the incidence of coronary heart disease and type 2 diabetes is due to its effects on insulin action and central adiposity.

2. High BMI. We have previously indicated that individuals with a high accumulation of visceral adipose tissue were characterized by metabolic alterations constituting the so-called metabolic syndrome or syndrome X, predictive of an increased risk for the development of type 2 diabetes, dyslipidemia, and coronary heart disease (27, 29). Furthermore, the relationship of visceral adipose tissue to metabolic complications was shown to be independent of concomitant variation in total body fat (19, 22). In effect, when two groups of obese men, matched for age and total body fatness, but having either a low ($109.9 \pm 14.0\ cm^2$) or high visceral fat area ($212.2\ cm^2 \pm 23.7\ cm^2$) determined by CT, were compared with a group of lean controls in the same age range and abdominal visceral adipose tissue area of $79.4 \pm 30.6\ cm^2$, it was reported that obesity *per se* (in the absence of a large accumulation of visceral fat) was associated with normal glucose tolerance and with trivial differences in plasma lipoprotein levels compared with lean controls (19, 22). However, the obese individuals with a large accumulation of visceral adipose tissue showed higher glycemic and insulinemic responses to an oral glucose challenge as well as a marked deterioration of their lipoprotein profile characterized by higher fasting plasma triglyceride levels and significantly lower plasma HDL cholesterol levels, mostly attributable to a decrease in cholesterol content in HDL 2 fraction (22–24) compared with the group of lean men. The group of obese men with low levels of visceral adipose tissue also showed a systematic trend for altered plasma lipoprotein-lipid levels compared with lean control subjects, but differences did not reach statistical significance (19). In another study comparing the fasting metabolic profile of middle-aged men with a wide range of BMI with low and high visceral adipose tissue accumulation, matched on the basis of total body fat mass, it was observed that the individuals with high levels of visceral adipose tissue area had higher mean plasma cholesterol and triglyceride levels and lower HDL cholesterol and FFA values as well as higher basal insulin and glucose levels, the differences in the fasting cholesterol and insulin being statistically significant (95). Visceral adipose tissue area explained the largest portion of the variance in plasma glucose and insulin responses to oral glucose while the abdominal to femoral adipose tissue areas (measured by CT) ratio explained the largest amount of variance in fasting plasma insulin and lipoprotein-lipid levels in the study in obese men by Pouliot *et al.* (19). LDL cholesterol levels were only marginally raised in visceral obesity (22–24, 29). However, by measuring apo B concentrations in the LDL fraction simultaneously or by separating LDL particles on the basis of their size by density gradient PAGE, Després (27) found that the dyslipidemic state of visceral obesity is accompanied by a greater proportion and by an increased concentration of small dense LDL particles. These changes in composition and concentration of LDL subfractions could not be detected by LDL cholesterol measurements. The presence of an increased proportion of small, dense LDL particles has been related to an increased risk for coronary heart disease (301). In a sample of 79 men, it was demonstrated that the dense LDL phenotype was characterized by increased plasma triglyceride levels, reduced HDL cholesterol, higher fasting insulin values, and elevated visceral adipose tissue area. It was concluded that the high triglyceride-low HDL cholesterol dyslipidemia frequently found in visceral obesity and in hyperinsulinemia is a strong correlate of the small dense LDL phenotype. Although associated with the dense LDL phenotype, visceral obesity and hyperinsulinemia were not independent predictors of an increased proportion of small dense LDL particles after controlling for triglyceride and HDL cholesterol levels (302). The same group demonstrated that the deterioration of the plasma lipoprotein profile observed in middle-aged men as compared with young adult men was partly mediated by a concomitant increase in total body fat and abdominal visceral adipose tissue (303).

In a sample of 52 obese premenopausal women, the amount of visceral abdominal fat, determined by CT, correlated with glucose tolerance during an oral glucose load, and after control for total adipose tissue mass, visceral fat accumulation remained significantly associated with glucose tol-

erance. As found in males, obese women with low levels of visceral fat (107.0 ± 33.4 cm^2) did not display glucose intolerance, and those with high levels of visceral fat (186.7 ± 36.9 cm^2) were observed to have the aberrations in glucose metabolism associated with the high adipose tissue mass, similar in both groups of obese women (18). However, in contrast to glucose tolerance, levels of plasma insulin and C-peptide areas were not correlated with the accumulation of visceral abdominal fat after control for total body fat, contrary to that found in men, as indicated above. Furthermore, the amount of visceral abdominal fat was an important correlate of the adverse changes in plasma lipoprotein-lipid changes, similar to that observed in abdominal obesity in men (304).

As indicated earlier, Bonora *et al.* (268) studied the relation of total body fat content and fat topography with *in vivo* glucose metabolism in obese premenopausal women. They did not observe any significant relationship between total body fat and any measure of insulin-mediated glucose metabolism, while visceral adipose tissue accumulation was the primary determinant of tissue sensitivity to insulin, being inversely related to total, oxidative, and nonoxidative glucose disposal during the insulin clamp.

To verify if gender differences in prevalence of cardiovascular disease risk factors could be explained by the level of visceral adipose tissue, Lemieux *et al.* (305) studied 80 men and 69 postmenopausal women, aged 23–50 yr. Despite the fact that women had higher levels of total body fat, they displayed lower areas of abdominal visceral adipose tissue and a lower ratio of abdominal visceral to midthigh adipose tissue areas than men. After adjustment for body fat mass, women generally displayed a more favorable risk profile than men, which included higher plasma HDL 2 cholesterol and lower plasma insulin, apolipoprotein B, and triglyceride levels. When the metabolic variables adjusted for body fat mass were compared between genders after control for differences in abdominal visceral adipose tissue area, variables related to plasma glucose-insulin homeostasis were no longer significantly different between men and women. Gender differences for plasma concentrations of triglycerides, apolipoprotein B, and the ratio of HDL 2 cholesterol/HDL 3-cholesterol also disappeared, whereas plasma concentrations of HDL cholesterol, HDL 2 cholesterol, as well as the ratio of HDL cholesterol/total cholesterol remained significantly higher in women than in men. These results suggested that abdominal visceral adipose tissue is an important correlate of gender differences in cardiovascular risk. However, additional factors were considered likely to be involved in gender differences in plasma HDL cholesterol levels.

B. Decrease in abdominal visceral fat: congenital generalized lipodystrophy

It is well known that generalized lipodystrophy is characterized by extreme paucity of subcutaneous and other adipose tissues, including intraabdominal omental, mesenteric, and retroperitoneal areas, as demonstrated by necropsy studies (306, 307), and by whole body MRI (308). However, the mechanical adipose tissue, which is relatively inactive metabolically and functions mainly in supportive or protective roles, as in the orbits, palms and soles, scalp, perineum and periarticular regions, among others, was detected (308). In addition, these patients as adults are characterized by insulin-resistant diabetes, hypertriglyceridemia, and muscular hypertrophy, masculine body build, acromegaloid stigmata, and organomegaly, as well as enlarged genitalia in infancy (309).

The results of body composition by dual-energy x-ray absorptiometry (DEXA) and abdominal fat areas by CT evaluation in our three female adult patients with congenital generalized lipodistrophy with insulin-resistant diabetes mellitus and severe hypertriglyceridemia are presented in Table 5. As can be seen, the patients with normal BMI presented an increase in lean body mass and a great reduction in body fat, as expected. Visceral abdominal fat detected in two of the patients was below the normal range for females while subcutaneous abdominal fat was absent, confirming the results obtained by Garg *et al.* (308). From all imaging studies, including ours, and the autopsy findings, it could be postulated that the genetic defect in congenital generalized lipodistrophy may result in poor growth and development of metabolically active adipose tissue whereas mechanical adipose tissue is preserved, as suggested previously by Garg and associates (307, 308).

As indicated in the *Introduction*, it was shown that, independent of total body fat, the visceral fat area evaluated by CT is associated with a decrease in insulin sensitivity measured by a euglycemic hyperinsulinemic clamp and is an important link between the several aspects of the metabolic syndrome. Thus, severe insulin resistance should not be expected to occur in congenital generalized lipodistrophy. An explanation for the presence of insulin resistance in the condition being discussed is that in humans, muscle triglyceride stores, as measured in biopsy samples, are inversely correlated ($r = -0.53$; $P < 0.0006$) to the insulin sensitivity determined by the euglycemic clamp. In turn, all measures of

TABLE 5. Body composition by dual-energy x-ray absorptiometry (DEXA) and abdominal fat areas (subcutaneous and visceral) by CT in three female adult patients with generalized congenital lipodystrophy

Initials	Age (yr)	BMI (kg/m^2)	BW (kg)	Lean body mass		Fat mass		Bone mineral content (kg)	Abdominal fat areas (cm^2)	
				kg	%BW	kg	%BW		Visceral	Subcutaneous
L.O.M.	20	19.4	48.0	43.4	90.4	3.0	6.3	2.04	0	0
A.M.A.	20	22.2	63.1	56.1	88.9	4.0	6.4	2.91	9.4	0.1
M.J.S.	36	21.5	49.2	44.0	89.4	3.0	6.0	2.20	10.3	0
Normal[a] female values	30.7 ± 6.4	21.2 ± 1.1	53.7 ± 4.7	36.5 ± 3.9	63.5 ± 4.5	18.7 ± 2.8	32.5 ± 4.4	2.20 ± 0.2	28.3 ± 17.5	128.4 ± 55.6

Data are mean ± SD.

[a] [Reproduced with permission from B. L. Wajchenberg *et al.*: *J Clin Endocrinol Metab* 80:2791–2794, 1995 (310). © The Endocrine Society.].

obesity were related to the insulin resistance measures independent of muscle triglyceride (311). Simoneau *et al.* (312) found that measurements of muscle fat and visceral obesity were independent predictors of insulin resistance. Moreover, stepwise regression revealed that an increase in muscle fat had the strongest predictive value for insulin resistance and, together with visceral fat content, accounted for 57% of the variance in glucose storage in the leg muscle that they analyzed. The parallels between these two sets of findings (311, 312) emphasize the dual but independent roles of muscle triglyceride and visceral adiposity, *i.e.*, possibly contributing through their influences on circulating lipids in insulin-mediated glucose uptake. Furthermore, in humans the inverse correlation of insulin sensitivity and muscle triglyceride is much stronger when intramyocellular fat is the measured variable ($r = -0.78$, $P < 0.0001$) (313). The same authors measured intramyocellular and extramyocellular fat content in the gastrocnemius/soleus complex of four patients with congenital generalized lipodystrophy with the use of magnetic resonance proton spectroscopy (314) and found that intramyocellular fat was significantly increased compared with normal controls while the extramyocellular lipid content was absent. Thus, it was concluded that the intramyocyte triglyceride content might be a factor in the genesis of their insulin resistance, explaining the finding of severe insulin-resistant diabetes in that disease in the absence or severe reduction in visceral fat mass.

Our finding of a very low LPL but normal hepatic lipase activity in post-heparin plasma in our three patients with congenital generalized lipodystrophy (B. L. Wajchenberg, unpublished data) could explain the high levels of circulating triglycerides and intraperitoneal fat, as shown by Stein *et al.* (313), having indicated that insulin resistance is closely related to skeletal muscle LPL activity (315) or low LPL in postheparin plasma (316). Further, it was suggested that a long-term exposure of the β-cell to excessive triglycerides might be an important factor in the dysfunction of the islets (261, 317). In conclusion, it can be suggested that the decrease in LPL activity associated with specific fat distribution in generalized lipodystrophy (lack of metabolic fat), either directly or via some metabolic abnormality, would lead to impaired hydrolysis of triglycerides in chylomicrons and VLDL in the circulation and in the muscle. This leads to insulin resistance and fat deposition in the islets, initially inducing hypersecretion of insulin to compensate for insulin resistance in the muscle and subsequently to β-cell failure associated with amyloid deposition, as shown in animals after the consumption of increased dietary fat (318) and described by Chandalia *et al.* (307) in a postmortem study of a patient with congenital generalized lipodystrophy. However, linkage analysis in ten consanguineous families with congenital generalized lipodystrophy ruled out mutations in genes related to lipid metabolism (LPL; apolipoproteins CII, AII, and CIII, hepatic lipase; hormone-sensitive lipase: β_3-adrenergic receptor; leptin and fatty acid-binding protein 2) as being involved in that pathological state (319). Recently, Garg *et al.* (320) analyzed 17 pedigrees with congenital generalized lipodystrophy and identified two loci for that disease, one on chromosome 9q34 (CGL1), which harbors a plausible candidate gene encoding the retinoid X receptor α (RXRA), which plays a central role in adipocyte differentiation. The other locus CGL2, unlike the 9q34 region, was not mapped.

VIII. Endocrinological Regulation of Abdominal Visceral Fat

A. Cortisol: Cushing's syndrome and visceral obesity

Cushing's syndrome is characterized by redistribution of fat from peripheral to central parts of the body, mainly in the abdominal region. Some obese patients have a Cushing-like appearance with typical adipose tissue distribution, including a preponderance of central fat. As a result, the clinical distinction between primary obesity and Cushing's syndrome is not always easy. We previously showed that female patients, aged 24–53 yr, with Cushing's disease presented a highly significant increase in total body fat and especially abdominal subcutaneous and visceral fat components, evaluated by CT at the level of the umbilicus, in comparison with lean female controls in the same age range. When the hypercortisolemic subjects were compared with obese individuals with the same anthropometric values (age, body weight, BMI, and WHR), whose visceral and abdominal fat areas were above our cut-off values (Table 2), the visceral adipose tissue area was significantly greater (196.9 ± 54.9 *vs.* 88.5 ± 47.1 cm^2) in endogenous hypercortisolemic individuals but not the subcutaneous abdominal area (309).

Because of the findings discussed above, the hypothalamic-pituitary-adrenal axis, particularly in visceral obesity, has been extensively evaluated. The studies have shown the following:

1. Increase in cortisol clearance. It was demonstrated that cortisol clearance (both absolute and body-weight corrected) showed a significant correlation with intraabdominal fat area, either expressed by WHR or obtained by CT. Thus, obese subjects with intraabdominal fat areas equal or greater than 107 cm^2 with an increased cardiovascular risk profile (Table 3) presented, as expected, a significantly higher cortisol clearance than the ones with areas lower than 107 cm^2 (80). The noncompartmental pharmacokinetic analysis of the cortisol data indicated that the volumes of distribution during the elimination phase and at steady state were higher in the visceral obese patients although nonsignificantly. The ratio of visceral/subcutaneous fat areas presented a significant correlation with the volume of distribution of cortisol at steady state (80), probably related to the larger number of glucocorticoid receptors in the adipocytes of the intraabdominal fat (117). There is the possibility that the increased number of glucocorticoid receptors could be responsible for a hypersensitivity of the intraabdominal fat adipocytes to cortisol, leading to accumulation of visceral adiposity. The increased MCR of cortisol is the probable explanation for occasional low plasma cortisol concentrations in samples collected at 0800 h in women with high WHRs, despite the increased cortisol secretion observed in those patients (321).

2. Increased 11β-hydroxy reductase activity in omental fat, generating active cortisol from cortisone. The expression of this enzyme being increased further after exposure to cortisol and insulin would ensure a constant exposure of glucocor-

ticoid to omental (but not subcutaneous) adipose tissue, promoting visceral obesity (322). In addition, it was demonstrated that in obesity, inactivation of cortisol by 5α-reductase is enhanced, as might be expected since fat contains 5α- but not 5β-reductase, while in the liver both enzymes are present (323).

3. Increase in urinary free cortisol, which was shown to be correlated with anthropometric parameters of visceral fat distribution, suggesting that cortisol production rate may increase as the amount of visceral fat enlarges (321).

4. Abnormalities of ACTH pulsatile secretion specifically higher than normal ACTH pulse frequency and reduced pulse amplitude, particularly during the morning, but with no change in cortisol parameters (324).

5. Sustained cortisol release regardless of its pulsatile rhythm after high protein and high lipid ingestion, particularly at noon (324).

6. Hyperresponsiveness of the hypothalamic-pituitary-adrenal axis (ACTH and/or cortisol) to different neuropeptides and environmental conditions. Specifically, this alteration was characterized, in obese premenopausal women, with visceral obesity, by a higher cortisol response to ACTH administration than obese subjects with subcutaneous body fat distribution and control normal-weight women (321, 325), as well as by an exaggerated ACTH and cortisol response to intravenous CRH alone or combined with AVP (arginine-vasopressin) and by higher than normal cortisol response to acute physical and mental laboratory stress tests (321, 326, 327). In addition, a decrease in the inhibition of cortisol secretion by very low doses of dexamethasone administration and an inverse correlation between the decrease in serum cortisol and the WHR has been described in men (328). These results, taken together, suggest that the HPA axis is hypersensitive and/or hyperactive in visceral obesity. The mechanisms responsible for these abnormalities are still unclear. However, several data support the hypothesis that central catecholaminergic and serotoninergic dysregulation may play a key role. In addition, they may represent part of an altered response to acute and/or chronic stress, which can be independent of the mechanisms responsible for feedback regulation. This is supported by both animal and human epidemiological data (324).

With respect to the effect of cortisol in the accumulation of visceral fat, as observed in Cushing's disease (310), the enhanced cortisol secretion resulting from a hyperactive HPA axis, an important pathogenetic factor for abdominal obesity, in the presence of hyperinsulinemia, associated with the well known state of insulin resistance in hypercortisolism (and visceral obesity) and the decrease of GH levels, as described in excess of glucocorticoids (329), would increase LPL activity and decrease lipolytic activity (34). The net effect would be expected to result in lipid accumulation. Because the density of the glucocorticoid receptors is higher in visceral than in other adipose tissues and remains so after exposure to excess cortisol (34) and the increased 11β-hydroxy reductase activity in omental fat, which generates cortisol from cortisone, as indicated above, the lipid-accumulating effect of cortisol would be more pronounced in visceral than in other fat areas. Thus, the findings in visceral obesity of enhanced cortisol production, hyperinsulinemia, and diminished GH secretion are in agreement with those of increased visceral fat in Cushing's syndrome, although the hormonal changes are of different magnitudes (34).

B. Testosterone

1. Testosterone in men. It was shown that visceral fat in men is strongly and negatively correlated to plasma total and free testosterone and sex-hormone binding globulin (SHBG) concentrations (330, 331), the latter attributed to the increased levels of insulin and/or IGF-I (332, 333). In young men, whose plasma total testosterone and free testosterone are high, the amount of intraabdominal fat is low, whereas as men age and their total and free testosterone decrease, more fat is deposited intraabdominally (334). The SHBG is also low, suggesting that a greater proportion of the total testosterone is free. Since the total testosterone is low, so is the free, even though less is bound. In moderately obese men, testosterone levels are decreased, a consequence of the decreased SHBG-binding capacity. Free testosterone levels, however, are normal as were LH levels, suggesting a normal hypothalamic control of LH secretion. In morbidly obese men (BMI $>$ 40), total and free testosterone and FSH and LH levels were decreased, indicating a functional impairment of the gonadostat responsible for the decreased free testosterone and hypogonadism (335, 336). This suggests a syndrome of hypogonadotropic hypogonadism only in the most obese men.

On the other hand, after adjusting for age and total fat mass, Leenen *et al.* (337) were unable to find significant associations between sex steroid levels and visceral adipose tissue mass as measured by MRI in obese men with BMI of 32.7 ± 5.1 kg/m^2 (mean $\pm$ SD). Similarly, Rissanen and coworkers (338), while studying obese men with a BMI of 31.8 ± 3.8 kg/m^2 (mean $\pm$ SD) using multislice MRI to measure visceral adipose tissue, were unable to relate visceral fat mass to free testosterone, total testosterone, or SHBG. Thus, visceral adipose tissue mass may be related to androgen levels, but this is still controversial, and the studies must take into consideration the fact that total body adiposity co-varies with visceral fat mass (339). Since estrogens are increased in obese men (181), they could be the explanation for the attenuated LH pulse amplitude with normal LH pulse frequency observed in morbidly obese men (340). As shown by Gooren (341), the administration of estrogen to nonobese agonadal men (male to female transsexuals) suppresses basal and GnRH-stimulated LH levels and LH pulse amplitude while LH pulse frequency remained unaffected. On the other hand, when given the antiandrogen, tamoxifen, there was a significant rise in the mean serum LH, which was associated with a significant increase in LH pulse amplitude but not LH pulse frequency and an increase in GnRH-stimulated LH response. However, tamoxifen administration to normal men resulted in a significant increase in mean LH values, with a significant increase in LH pulse frequency and amplitude (342).

In a study of ten young morbidly obese men, presenting low total and free testosterone and slightly increased estradiol but high estrone values, with low basal LH and decreased GH response to 100 μg of GnRH iv, the administra-

tion of tamoxifen, for 1 month, was associated with an increase in total and free testosterone levels, as well as mean LH, normalization of LH pulse amplitude, but no change in frequency, and increased response to GnRH injection (B. L. Wajchenberg, unpublished data). A possible explanation for the discrepancy in the effects of the antiestrogen in morbid obesity and normal men could be the interaction between estrogens and testosterone on gonadotropin secretion in the latter while the interaction between low androgen and high estrogen could explain the impaired regulation of the gonadostat in massively obese men, characterized as mentioned by decreased LH levels and pulse amplitude, and responsible for the decreased free testosterone levels, as suggested by Giagulli *et al.* (336).

It should be emphasized that the statistical correlations that have been found between low circulating androgens on the one hand and abdominal visceral obesity on the other do not address the issue of causality in the fatness-steroid associations. Schneider *et al.* (181) suggested that the reduced plasma testosterone levels in obese men are due to an increased clearance rate rather than to a reduced testosterone production, the expanded adipose tissue mass representing a major site of testosterone conversion into estrogens, in accordance with higher estrone concentrations and reduced testosterone and adrenal C 19 levels in moderately obese men as compared with lean controls (182).

Märin *et al.* (343) treated middle-aged men with abdominal obesity with transdermal testosterone, resulting in elevated testosterone concentrations and marked decreases in FSH and LH. Visceral fat mass decreased without significant changes in other depot fat regions while lean body mass did not change. Glucose disposal rate, measured with the euglycemic hyperinsulinemic clamp, increased markedly while fasting plasma glucose, cholesterol, triglycerides, and diastolic blood pressure decreased. It should be pointed out that these subjects were not truly testosterone deficient, but rather in the low-to-medium normal range. The authors suggested that testosterone might work by decreasing LPL activity and improving lipolysis in visceral adipocytes, which will be discussed below.

Visceral fat has a high density of androgen receptors and testosterone and, as previously indicated, amplifies its own effect by up-regulation of androgen receptor number, inhibiting the expression of LPL and FFA uptake in the omental but not femoral adipose tissue (34, 118). Simultaneously, catecholamine-induced lipolysis is markedly stimulated at the levels of β-adrenergic receptors, whose density is up-regulated, as well as adenylate cyclase, protein kinase A, and hormone-sensitive lipase activity, whereas G-proteins seem unaffected (34). The presence of GH is of major importance for fully expressed testosterone actions (344). The net effect is lipid mobilization, resulting in a diminution of visceral fat mass and a decrease in omental adipocyte size associated with decreased leptin expression and secretion, there being an inverse relationship between free testosterone levels and plasma leptin ($r = -0.57$, $P < 0.001$) (118). These effects of testosterone on adipocyte metabolism would be expected to markedly inhibit triglyceride uptake and stimulate their mobilization seen in *in vivo* studies in men, suggesting that testosterone is an important regulator of the proportion of depot fat mass in central and peripheral adipose tissue in human males (345).

It is possible that the negative relationship between visceral fat mass and testosterone is related to the effect of the steroid in increasing muscle insulin sensitivity preceding the decrease in visceral fat mass (346). However, testosterone exerts optimal effects on insulin sensitivity within a range of near-normal testosterone concentrations; apparently, both excessively low and excessively high levels are associated with insulin resistance (34). It is likely that the GH deficiency and excess of cortisol secretion seen in visceral obesity contribute to the distribution of body fat to visceral adipose tissue, counteracting the effects of testosterone.

2. Testosterone in women. It has been reported that in women, visceral obesity is associated with elevated levels of total testosterone, free testosterone, and a reduction in SHBG (347). While in obese men, Leenen *et al.* (337) were unable to observe a significant correlation between androgen levels and visceral adipose tissue measured by MRI, in women with a similar BMI, an abundance of visceral fat was associated with declining levels of SHBG and elevated levels of free testosterone but not changes in total testosterone after adjustment for age and total fat mass. These observations and the fact that female-to-male transsexuals treated with testosterone have an increase in visceral fat only when oophorectomized (119), as previously indicated, would indicate that testosterone causes accumulation of visceral adipose tissue consistent with the suggestion that testosterone might be a factor for the visceral fat accumulation in hyperandrogenic women. However, as seen by the documented effects of testosterone on LPL activity and lipolysis mentioned above, testosterone would be expected to diminish visceral fat depots. One possible explanation is the role of increased production of estrogens in obesity, particularly in postmenopausal women. Another is the observation that visceral fat increments occur only in transsexual women who have had an oophorectomy (119), suggesting that the remaining estrogen production before the oophorectomy (341) was protective. The androgen receptor in female adipose tissue seems to have the same characteristics as that found in male adipose tissue whereas estrogen treatment down-regulates the density of this receptor (34), which might be a mechanism whereby estrogens protect adipose tissue from androgen effects. Estrogen by itself seems to protect postmenopausal women from visceral fat accumulation (121). Therefore, when estrogen levels become sufficiently low, visceral fat accumulation may occur. The balance between androgens and estrogens therefore seems of significance; perhaps the lack of estrogen is more important than the relatively small androgen excess in hyperandrogenic women with visceral obesity. It is also possible that hormones other than androgens and estrogens exert powerful effects on hyperandrogenic women, overriding those of androgens. As indicated for males, elevated cortisol production in visceral obesity and low GH in that condition can be expected to increase visceral fat content.

C. GH

In obesity, there is a decrease in circulating GH and a blunted response to provocative testing (347). In healthy obese middle-aged men, with a BMI ranging from 28 to 33 kg/m^2, a double defect in GH dynamics was shown. This was characterized by a reduction in daily GH production rate and accelerated disposal rate not due to decreased circulating levels of GH-binding protein, which are similar in obese and normal weight controls. There was a strong inverse relationship of BMI to GH pulse frequency and GH production rate (348). Using a specific and ultrasensitive assay, Rasmussen *et al.* (349) confirmed the findings discussed above, except that the reduction in the number of GH peaks in obese compared with normal subjects was not observed, probably due to the fact that in the study by Veldhuis and co-workers (348), conventional RIAs were used and many serum GH concentrations were immeasurably low and may have underestimated GH secretory burst frequency. Levels of IGF-I in obesity have been variously reported to be increased, normal, or decreased (339). The hyperinsulinemia associated with obesity decreases IGFBP-1, which may account for the majority of studies of obese adults and children demonstrating a decreased total IGF-I level (350). Interestingly, subjects with visceral obesity have lower total IGF-I levels independent of total fat mass (334, 351). This is most likely a result of the positive relationship between visceral adipose tissue and fasting insulin levels and, hence, lower IGFBP-1 levels. Similarly, Rasmussen *et al.* (349) have demonstrated an inverse correlation between 24-h GH secretion or total IGF-I and visceral adipose tissue in obese and lean adults. Frystyk and co-workers (350) have shown that free IGF-I levels are increased in obese men and to a lesser extent in women, which may, by feedback, explain the decline in GH secretion with increasing body fatness and may be responsible for the normal growth without GH in obese children. The source of the increased free IGF-I may be the production of IGF-I by adipose tissue. In effect, several studies have shown IGF-I mRNA and protein in adipose tissue and that IGF-I is a potent factor in the proliferation and differentiation of adipose tissue that plays an important role in the regulation of adipose tissue (238, 352). However, definitive evidence for the release of IGF-I from adipose tissue *in vivo* is lacking. As indicated by Smith (339), given the mass of the adipose tissue in obese individuals and the IGF-I present and presumably secreted, adipose tissue could be a major source of IGF-I in excess of that produced by the liver. The reversibility with weight loss strongly suggests that the alterations in the GH-IGF-I axis are secondary to the obese state and not causative (350).

The involvement of GH in the regulation of visceral fat mass in humans is clearly demonstrated by the observation that in acromegaly there is a reduction in visceral adipose tissue (353). When such patients or subjects presenting other pituitary tumors have pituitary surgery and/or irradiation and are given replacement therapy (except GH), visceral fat increases significantly, and after administration of GH, a marked reduction of visceral fat mass occurs (354, 355). Most of the actions of GH on adipose tissue are to prevent lipid accumulation and to stimulate lipid mobilization, which requires synergism with steroid hormones. With respect to the lipid accumulation, LPL is markedly inhibited by GH in the presence of either testosterone or cortisol (34). After lipid mobilization, GH exerts intense stimulatory effects, and it has been shown that glucocorticoids and thyroid hormones are important for the GH effects on lipolysis (34). Furthermore, GH enhances catecholamine-induced lipolysis via β-adrenergic receptors (356). The levels of regulation of lipolysis are multiple, including β-receptor density and adenylate cyclase as well as protein kinase- and/or hormone-sensitive lipase activities (34). From the results of several studies at the cellular and molecular levels and in humans, it can be concluded that GH exerts a permissive effect on both LPL and lipolysis. Its occurrence mainly in visceral adipose tissue might be attributable to the fact that cellular mass, blood flow, and innervation are higher in this than other adipose tissue sites (357, 358), which would make the hormonal interactions with adipocytes more pronounced here than in other regions. Furthermore, since GH actions are dependent on steroid hormone interactions and their receptors are particularly dense in visceral adipose tissue, as previously indicated, a more pronounced effect of GH would be expected in this tissue.

D. Estrogens

Clinical observations indicate that female sex steroid hormones are involved in the determination of body fat distribution, *i.e.*, greater accumulation of subcutaneous fat in the gluteofemoral region (14) and less visceral fat mass than men (55). The female distribution of body fat tends to disappear, at least partially, with the menopause inasmuch as women tend to accumulate visceral fat that can be prevented by hormonal replacement therapy (121). In effect, women with normal ovarian steroid production have a higher LPL activity and LPL mRNA in the femoral than in the abdominal fat cells (103) while the lipolytic process shows the opposite relationship, the catecholamine-induced rate of FFA mobilization from visceral fat being lower in women than in men (116). The net effect is that normal, premenopausal women tend to accumulate triglycerides in the gluteofemoral region (359). With menopause, the functional pattern of different adipose tissue in women changes: LPL activity decreases and lipid mobilization becomes more amenable to stimulation in the gluteofemoral depots (34). In the reversion to a premenopausal fat pattern with hormonal replacement therapy, estrogen is the most active component, although additional effects of progesterone cannot be excluded (360). The possible mechanisms for the effects of estrogens on the determination of body fat distribution include the down-regulation of the androgen receptor, thereby preventing androgen effects (34) as mentioned earlier; an indirect effect by increasing GH secretion, estrogen receptor-mediated primarily acting at the hypothalamus (361). Probably both mechanisms may be involved. Regarding the physiological relevance of the estrogen receptors in human adipose tissue (122), it has not yet been clearly established. In relation to progesterone it competes with cortisol binding to the glucocorticoid receptor (123) and may therefore protect from cortisol effects.

In conclusion, the hyperandrogenicity, low GH levels, and

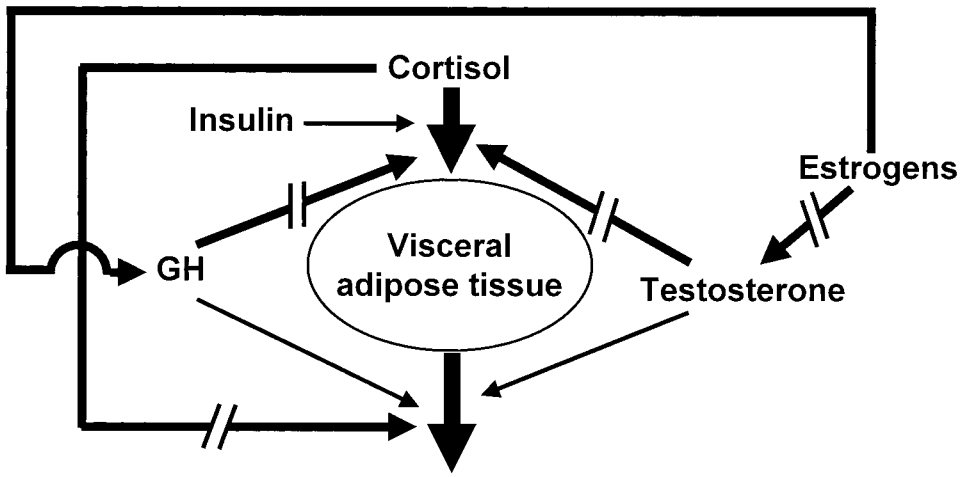

FIG. 6. Hormonal regulation of abdominal visceral fat. [Derived from Ref. 34.]

increased cortisol secretion frequently seen in women with visceral obesity may be involved in the centralization of body fat. Figure 6 summarizes the hormonal regulation of abdominal visceral fat.

IX. Summary

Recent studies have demonstrated that regional distribution of adipose tissue is critical in the clinical assessment of patients, particularly if they are obese. In effect, excess fat in the central (visceral abdominal) *vs.* peripheral part of the body (gluteofemoral) independent of overall obesity is associated with higher plasma glucose and insulin, hyperlipidemia, and decreased HDL cholesterol concentrations, components of the insulin resistance syndrome and constituting a cluster of risk factors for atherosclerotic cardiovascular disease, as shown in prospective studies. To assess the abdominal visceral fat, the best of the anthropometric indexes are the measurement of waist circumference or abdominal sagittal diameter, both presenting excellent correlation with the gold standard, CT or MRI of the abdomen. Another method, the measurement of intraabdominal thickness by ultrasound has also shown good correlation with visceral fat by CT.

The amount of visceral fat increases with age in both genders, males having greater mass of visceral adiposity. Regarding the total body fat, the correlation was low or moderate with CT-determined visceral adipose tissue. Positive energy balance is associated with intraabdominal fat accumulation but it is not a strong determinant. Adipose tissue LPL activity is high in the depot as well as the lipolytic activity. α_2, β_1, -2, And -3 adrenoreceptors and receptors for insulin, adenosine, glucocorticoids, and testosterone are present in visceral fat, having a major functional role. The receptors for GH, thyroid hormones, and estrogens have a role not yet completely elucidated.

The genetic effect on visceral fat (adjusted for fat mass) is about 50% of the phenotypic variance. Recent studies suggest that a great number of genes, loci, or chromosomal regions, distributed on the different chromosomes, could play a role in determining body fat and fat distribution in humans.

Adipose tissue releases or expresses a variety of peptides and nonpeptide compounds, such as LPL, which is induced in the visceral fat by glucocorticoids; acylation stimulated protein acting in sequence to LPL; cholesterylester transfer protein activity, which is increased in omental adipose tissue; RBP; plasminogen activator inhibitor-1 produced more in the visceral than in subcutaneous fat and presented a strong correlation with the insulin resistance syndrome and the risk of macrovascular disease. Among the secreted factors with an endocrine function, the following should be mentioned: estrogens, resulting from aromatization of androgens by the adipose tissue, stimulated by insulin and cortisol; leptin, reflecting fat hypertrophy, the major source being the subcutaneous rather than visceral fat (due to the larger size of the subcutaneous adipocytes), being regulated by factors other than the size of the adipose tissue, such as energy balance and hormones (glucocorticoids and insulin) and tumor necrosis factor-α modulating positively leptin secretion; angiotensinogen, which via angiotensin II, produced locally, induces differentiation of preadipocytes into adipocytes; and adiponectin, which is inversely correlated with body weight. Among the factors with an autocrine/paracrine activity regulating adipose tissue cellularity, the adipocytes are a source and target tissue of TNFα, which induces insulin resistance, expressed equally in the different fat depots, being a regulator of fat cell size. Another factor is PPAR-γ, a ligand-activated transcription factor that, when activated, promotes differentiation of preadipocytes into mature adipocytes from subcutaneous but not intraabdominal fat, antagonizing TNFα-induced insulin resistance and preventing the anti-adipogenic effects of this cytokine. A third factor is interleukin-6, being released more by visceral (omental fat) than subcutaneous abdominal adipocytes being induced by TNFα. A fourth factor is IGF-I produced by the adipocyte, being a potent factor in the proliferation and differentiation of preadipocytes, acting in an autocrine/paracrine fashion. Regarding the UCPs, the reduction of UCP-1 and -2 mRNA in visceral fat is compatible with a decreased capacity to expend energy in visceral obesity. Finally, monobutyrin, secreted by the adipocyte, favors the vascularization of adipose tissue.

The higher lipolytic activity in visceral fat in comparison with other depots, related to the increased expression of β-adrenoreceptors, particularly β_3, associated with a decrease in α_2-adrenoreceptor-dependent antilipolysis, results in higher FFA mobilization to the liver. The elevated FFA flux to the liver decreases hepatic insulin extraction, leading to systemic hyperinsulinemia as well as inhibiting the suppression of hepatic glucose production by insulin. In addition, FFAs accelerate gluconeogenesis and increased secretion of VLDL. In addition, the increase in FFAs decrease insulin-stimulated peripheral glucose disposal, which in normal subjects is compensated by FFA-induced potentiation of glucose-stimulated insulin secretion, determining still greater peripheral hyperinsulinemia. Chronic exposure to high levels of FFA contribute to β-cell failure and the development of type 2 diabetes.

Thus, subjects with visceral abdominal obesity are more insulin resistant than those with peripheral obesity, have lower glucose disposal during an euglycemic hyperinsulinemic clamp, reduced oxidative and nonoxidative glucose and leucine disposal, and significantly greater lipid oxidation. These results suggest that an excess of visceral fat results in a detrimental effect on glucose and protein metabolism, the excess of FFA being the link between central fat and insulin resistance.

Hyperinsulinemia and insulin resistance are common in nonobese Caucasian males, and the multiple risk factors for coronary heart disease are prevalent in such individuals, corresponding to the "metabolically obese" normal-weight subjects. Among the factors associated with this condition are an increase in visceral fat mass (antedating and accompanying the components of the metabolic syndrome) followed by insulin resistance caused by an increased flux of FFAs. Aging is another factor associated with an increase in visceral fat as well as low birth weight and low weight at l yr of age. Other factors to be taken into consideration are inactivity and decreased fitness (low $VO_{2\ max}$), since it is possible that central adiposity, low birth weight, and inactivity, in concert and independently, contribute to the development of insulin resistance in both normal-weight and obese individuals. Finally, the improvement in insulin sensitivity by regular exercise in individuals with visceral obesity and insulin resistance is associated with a disproportionate loss of visceral fat.

Whereas obese patients, in the absence of an abnormal accumulation of visceral fat, have normal glucose tolerance and lipoprotein levels compared with lean controls, those with a great increment in visceral adipose tissue present higher glucose and insulin responses to oral glucose. They also have higher fasting plasma triglycerides and lower HDL cholesterol levels (LDL cholesterol being only marginally raised with a great proportion) and increased concentrations of small dense LDL particles, which have been related to an increased risk for coronary heart disease. The dense LDL phenotype in visceral obesity is characterized by increased plasma triglycerides, reduced HDL cholesterol, and high fasting insulin. The gender differences in the prevalence of cardiovascular disease risk could be explained by the level of visceral fat, with women presenting lower levels of abdominal visceral adiposity than men, after adjustment for body fat mass. However, additional factors should be considered in gender differences in HDL cholesterol levels, which are higher in women.

The decrease in visceral fat, as seen in congenital generalized lipodystrophy, is associated with an increase in lean body mass and characterized by insulin-resistant diabetes and hypertriglyceridemia, which relates to an increase in intramyocellular fat, the strongest predictor for insulin resistance.

In abdominal visceral obesity, the multiple endocrine abnormalities found in obesity are more pronounced than in other obesity phenotypes, being characterized by an increase in cortisol production resulting from a hypersensitive and/or hyperactive hypothalamic-pituitary axis representing part of an altered response to acute and/or chronic stress. The increase in cortisol secretion in the presence of hyperinsulinemia, associated with the state of insulin resistance of the hypercortisolism, and the decrease in GH levels related to the excess of cortisol induce the increase in LPL and a decrease in lipolytic activities, resulting in fat accumulation similar to that found in spontaneous Cushing's syndrome.

Another hormone involved in visceral adipose tissue accumulation is testosterone, which is strongly and negatively correlated to visceral fat in men. Since SHBG is low due to the hyperinsulinemia of the obese state, the greater proportion of the total testosterone is free. Thus, in moderately obese men, testosterone levels are low and free fraction is normal as well as the hypothalamic-pituitary-testicular axis. In morbidly obese men, total and free testosterone and gonadotropin levels are decreased, indicating a functional impairment of the gonadostat, which is probably dependent on the higher estrogen levels related to the conversion of testosterone into estrogens by the expanded adipose tissue mass, further reducing the androgen levels. Testosterone might work by decreasing LPL activity and improving lipolysis in visceral adipocytes, thereby decreasing the visceral fat mass. Since GH is necessary for full expression of testosterone action, it is likely that GH deficiency and cortisol excess contribute to the distribution of body fat to visceral adiposity, thereby counteracting the effects of testosterone.

In women, visceral obesity is associated with elevated levels of total and free testosterone and low SHBG levels as observed in hyperandrogenic women. Because estrogens down-regulate the testosterone receptor density in visceral fat, it protects female adipose tissue from androgen effects. Therefore, estrogen levels with relatively small androgen excess in hyperandrogenic females can induce visceral fat accumulation, without discarding the important role of elevated cortisol production and low GH in visceral obesity.

GH secretion, as indicated, is greatly reduced in obesity, and low IGF-I levels in the latter are related to the hyperinsulinemia-associated reduced IGFBP-1 in visceral obesity but normal free IGF-I. The role of IGF-I produced in the adipose tissue should be taken into consideration. The alterations in the GH-IGF-I axis are secondary to the obese state and not causative. GH exerts a permissive effect on both LPL and lipolysis mainly in the visceral fat dependent on interaction of steroid hormones.

Finally, estrogens, by down-regulating the androgen receptor, tend to decrease visceral fat accumulation. Another

mechanism for the effects of estrogens on the determination of body fat distribution is an indirect one, i.e., acting on the hypothalamus and increasing GH secretion.

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