The Role of Fat Depletion in the Biological Benefits of Caloric Restriction\textsuperscript{1,2}

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ABSTRACT One of the most robust observations in the biology of aging is that caloric restriction (CR) extends life in a variety of species. Although CR results in substantial decrease in fat mass, the role of fat in life extension was considered minimal. Indeed, in the fields of obesity and diabetes, the amount of fat has been directly implicated in the metabolic consequences. Since it became apparent that fat is a massive endocrine tissue, some of its roles have been recently revised. Many of the systemic effects of CR can now be explained by the chronic effects related to decreased plasma levels of peptides, cytokines, complement factors and substrates that are produced in fat. Most of the benefits of CR on the neuroendocrine system and those related to the improvement in glucose homeostasis can be attributed to a decrease in adipose cells and their products. If all or most of the life-extending benefits of CR can be attributed to decreased fat stores, the expression of specific candidate substrates and proteins may be explored and manipulated in searching for the most powerful adipose-dependent signals that modulate life expectancy. J. Nutr. 131: 903S–906S, 2001.

KEY WORDS: • caloric restriction • fat mass • visceral fat • obesity • insulin sensitivity
• fat-derived peptides

Although change in body weight, specifically marked reduction in fat mass (FM)\textsuperscript{4}, is the main phenotypic expression of caloric restriction (CR) in animals, only a few studies in the last decades have examined the causal relationship of FM to different metabolic disorders. The most compelling reason for investigating the role of FM in life extension is the fact that CR has overwhelming effects on a variety of organs and systems. In both animals and humans, elevated total body FM causes an increase in morbidity and mortality that may be attributed to a variety of pathological factors, among which insulin resistance is most important. Peripheral and hepatic insulin resistance is characterized by marked increases in fasting and postprandial insulin levels and, consequently, impaired glucose tolerance. Moreover, although insulin resistance is common in aging, it has been shown to be determined by FM in both rats and humans. Because CR reverses the increase in plasma insulin, glucose and glycosylated hemoglobin to youthful levels, it has been hypothesized that glucose and insulin mediate aging processes (the glucose and insulin hypothesis). A recent study showed a dramatic increase in life span in a nematode with a mutation of an insulin-like receptor complex gene, further supporting the importance of the insulin-signaling pathway in longevity (1). As will be discussed, increased body weight and FM in humans results in insulin resistance and is closely associated with the development of dyslipidemia, hypertension, diabetes, and coronary heart disease, leading to early death.

**FM, CR and peripheral insulin sensitivity**

Most studies express glucose uptake in terms of kg of body weight, which does not correct for large amounts of relatively inactive adipose tissue and results in an underestimation of glucose uptake by lean body mass (LBM). Thus, while most studies demonstrated a decline in glucose utilization, body mass index (BMI) and FM were inversely correlated with glucose infusion rates during hyperinsulinemic clamp studies, independent of age (2,3). Indeed, insulin sensitivity in a large number of lean older individuals was similar to that of weight-matched young individuals (4). A recent multi-center study demonstrated that FM, and not age, caused decreased peripheral insulin sensitivity. Thus, FM has an overwhelming effect on peripheral insulin sensitivity with aging. Increased visceral fat (VF) was shown to be associated with a decrease in skeletal muscle insulin sensitivity measured by leg balance studies and

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\textsuperscript{4} Abbreviations used: FM, fat mass; CR, caloric restriction; LBM, lean body mass; BMI, body mass index; VF, visceral fat; HGP, hepatic glucose production; AT, angiotensinogen.

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a decrease in insulin responsiveness in BMI-matched women with upper versus lower body obesity (5,6). While improved insulin action is typical for CR and may be involved in mechanisms affecting longevity, impaired insulin action in humans is associated with morbidity and mortality. As in humans, rodents display an increase in basal plasma glucose and insulin levels with aging (7). The advantage in studying animal models is the ability to intervene by diet, drugs or surgery in a relatively homogeneous obese group and to relate the physiologic and molecular aspects of these changes.

We examined the relationship between FM, peripheral insulin and age in rats (8). We studied body composition and rates of (LBM) glucose uptake in a group of 2-, 4-, 6-, 12- and 18-mo-old rats. The results showed a significant gradual increase in FM from 2 to 18 mo of age (316% increase), with a significant increase in FM from 2 to 18 mo of age (316% increase), with a high correlation between body weight, FM and epididymal fat. The rate of insulin-stimulated glucose uptake decreased significantly (~14%) from 2 to 4 mo of age. Surprisingly, we found that there was no further change in insulin responsiveness in rats that were much older. Analyzing the data using a best fitted model of two regression lines joined in a knot (spline curve model) demonstrated that when body weight was ~350 g or ~14% fat, no change in glucose uptake was noted with further increased body weight. This analysis suggests that the effect of body weight was saturable for the decrease in glucose uptake. Using the same statistical methodology to analyze the relationship between glucose uptake and FM and epididymal fat showed similar results (8). Because this saturable effect occurred at a relatively young age, it indicates that aging per se is not associated with further decrease in peripheral insulin sensitivity. We next set to prove this concept by examining whether a cause-effect relationship exists between FM and age-related decrease in peripheral insulin responsiveness (plasma insulin levels of ~100 pmol/L). We used the same age-group animals as the above ad libitum-fed and compared them with chronically CR rats (Fig. 1). When CR young and older adult rats (2–4 mo) were studied, their insulin responsiveness significantly increased. At this time, their FM decreased to below 13% of their body weight. Interestingly, insulin responsiveness in older calorie-restricted animals did not improve; however, their FM percentage was higher (17%). We recently studied an additional group of more CR (50% food intake (18 mo; n = 5)) to decrease their FM to ~13%.

This resulted in a dramatic improvement in insulin responsiveness that has not been noted when CR resulted in a decrease from 27 to 17% in FM (Fig. 1). Saturating effects of FM on peripheral insulin action had been previously demonstrated in Pima Indians (9), suggesting that once a certain critical level of fat accretion is achieved, further alterations in glucose metabolism are difficult to detect. In addition, this suggests that chronic benefits of weight reduction may be obtained not by losing any weight but by losing weight below a certain amount of fat. Furthermore, this suggests that FM accumulation, rather than aging, is responsible for the age-dependent insulin resistance.

CR affects hepatic insulin sensitivity

It was previously demonstrated that fasting plasma insulin levels are increased in both humans and rodents with aging. This is due to impaired suppression of hepatic glucose production (HGP) requiring significant portal hyperinsulinemia (10). However, suppression of HGP by insulin was significantly impaired in younger subjects with increased abdominal obesity (11). To better examine this question, we used chronic CR to maintain FM and VF at youthful levels and to abolish the overwhelming negative effect of fat on hepatic insulin action in 8- and 20-mo-old Brown Norway Fisher rats (n = 344). Using the pancreatic clamp technique, we measured their basal HGP and by inducing mild physiological hyperinsulinemia, we measured the ability of insulin to suppress HGP (Fig. 2). The results demonstrated that basal HGP was similar in all groups. However, insulin-induced suppression of HGP was significantly increased in the calorie-restricted (8- and 20-mo-old) rats compared with their ad libitum-fed controls (46 and 39%, respectively). In parallel, the percentage of FM and VF was significantly and proportionally lower in the CR groups. Importantly, rats were chronically calorie-restricted from young adulthood to maintain body composition at youthful levels. This study demonstrates that insulin action on modulating HGP is not impaired by aging per se but by age-related increases in FM and VF. This experimental manipulation provided evidence to support the hypothesis that the capacity of insulin to regulate hepatic glucose flux is not impaired with aging (12).

Fat distribution with aging and its metabolic effects

Increased VF is a common and typical change in body composition with aging (13,14). The increase in VF can be achieved not by losing any weight but by losing weight below a certain amount of fat. Furthermore, this suggests that FM accumulation, rather than aging, is responsible for the age-dependent insulin resistance.

**Supplement**

**FIGURE 1** The effects of aging on insulin-mediated glucose uptake in ad libitum-fed and chronically calorie-restricted rats. Glucose uptake was markedly decreased after puberty and did not decrease with aging after late adulthood, when expressed in terms of lean body mass. CR restored insulin action when FM was decreased to below 13–14% of body weight. When tight CR was applied and FM decreased to 13%, glucose uptake increased dramatically. Values represent the mean ± SE.

**FIGURE 2** Suppression of HGP from basal in ad libitum-fed and calorie-restricted rats. Physiological hyperinsulinemia induced a more prominent suppression of HGP (with age) in calorie-restricted rats compared with ad libitum-fed rats; however, maximal suppression could not be achieved.
detected surgically (increased omental fat) or by CT scan or magnetic resonance imaging. However, the most common epidemiological tools are the observation of a pattern of upper body obesity, increased waist circumference and increased waist to hip ratio. Most important, waist to hip ratio in older men and women is increased independently of BMI (13). CT scan studies in men and women of all ages revealed a significant inverse correlation between the ratio of subcutaneous to VF tissue with age (14) and an increased accumulation of fat between the muscles of the abdominal wall in older compared with younger subjects (15). Thus, older men and women show an increase in VF, although the BMI may be normal and LBM may be decreased (16). VF has multi-factorial associations with aging and may determine longevity in humans by influencing atherosclerotic mechanisms (17). Numerous studies in subjects of all ages have demonstrated that increased VF is associated with increases in fasting and postprandial plasma insulin levels and impaired glucose tolerance (18,19).

This knowledge provided us with the opportunity to study specifically one small fraction of fat to prove the effects that fat has on the paradigm of ad libitum-fed and CR rats. Thus, removal of VF may provide some cause-effect relationship in the understanding of their metabolic impact. To directly examine whether VF modulates hepatic insulin action, we randomized moderately obese Sprague-Dawley rats either to surgical removal of the epididymal and perinephric fat pads or to sham operation and then studied them 3 wk later (20). Total VF was ~fourfold increased in the sham-operated group. However, whole body FM was not significantly different. While plasma glucose, FFA, glycerol and glucagon were similar, plasma insulin levels were decreased by one half in rats with plasma glucose levels to maintain plasma glucose levels at baseline during the hepatic-surgically removed VF. The rate of insulin infusion needed to decrease in the plasma levels of insulin-like growth factor binding protein-1, a marker of insulin's transcription regulation in the liver. The marked increase in hepatic insulin action after surgical removal of selective intra-abdominal fat depots indicates a cause-effect relationship between them and explains the basal hyperinsulinemia in aging. Subsequently, we generated similar decreases in VF using leptin or β-adrenoceptor agonists and demonstrated that hepatic insulin action was equally improved in all rats studied. While we defined specific effects to specific fat tissue (VF), these studies have also supported the general concept that fat has an overall metabolic effect (20).

Fat-derived peptides, their metabolic actions and risk factors for diseases

In the last several years, various peptides have been identified and shown to have the following characteristics: they are produced either primarily or also by fat tissue, secreted and measured in the plasma and have a distinct action on other tissues of organs. Additionally, manipulation at the level of FM (pharmacologically, CR or surgical removal of fat) induces important changes in these peptides action. The fat-derived protein TNF-α has been suggested to be directly involved in the development of insulin resistance in obesity by its effects on insulin signaling (21,22). We studied TNF-α gene expression in a variety of fat depots after surgical extraction of VF and compared the results with gene expression in sham-operated rats with intact VF (20). Most impressively, the gene expression of TNF-α in the subcutaneous fat (the largest fat depot) was decreased by ~fourfold to fivefold in rats without VF. Marked (45%) decreases in TNF-α gene expression have been demonstrated in fat obtained from obese humans who lost weight (23) and after dietary manipulations in obese mice. Leptin, the most investigated fat-derived peptide, also has tremendous implications in glucose metabolism (24); during physiological hyperinsulinemia (insulin clamp), leptin markedly enhanced the action of insulin on inhibiting HGP (~fourfold lower in leptin than in the control groups). This may have been through its effect on decreasing VF. However, leptin had an additional effect in improving peripheral insulin action; the rate of glucose uptake in the leptin group was 52 and 33% higher than in saline-administered and pair-fed groups, respectively. Interestingly, leptin may be responsible for many of the neuroendocrine adaptations to starvation and CR; during weight loss or fasting, both humans and animals (25,26) exhibit low levels of leptin gene expression and plasma levels. These conditions are associated with a marked neuroendocrine adaptation, including increased steroid stress hormones, decreased reproductive function, decreased thyroid function and delayed puberty (27). Angiotensinogen (AT), another peptide expressed in liver and adipose tissue (28), may also play an important role in the relationship between obesity, hypertension and hyperinsulinemia (the main components of syndrome X). The hormonal control of AT synthesis and secretion from adipocytes has been shown to be up regulated with glucocorticoids and TNF-α. However, in vitro studies have shown that insulin exerts an inhibitory effect on AT production (29). To examine the role of increased FM on AT, we followed AT expression after 3 h of hyperglycemia while maintaining plasma insulin concentration at basal (using somatostatin infusion) in lean and obese rats. The relative increase in AT expression was ~threefold in both groups (Fig. 3); however, the obese rats had ~fourfold increased FM compared with the lean group. Additionally, the obese rats also demonstrated resistance to the inhibitory effect of insulin on AT expression when examined in an hyperinsulinemic clamp.

FIGURE 3 Hyperglycemia and AT gene expression in lean and obese rats. Glucose was infused with somatostatin to increase plasma glucose levels to ~18 mM and to inhibit endogenous insulin secretion, for 3 h. AT gene expression was significantly (P < 0.001) increased from basal after glucose infusion in lean and obese animals.
These findings demonstrate a potential pathological link between fat and hypertension. Plasminogen activator inhibitor-1 is also secreted from fat tissue (30), is increased with obesity, is associated with decreased fibronectin activity in the blood and is a risk factor for acute coronary events in humans. In fact, moderate CR in elderly obese subjects decreases plasminogen activator inhibitor-1 levels dramatically (31). In addition to cytokines, complement factors (D, C3, B) are also secreted by fat tissue and have a role in controlling immune responses (32,33). Newly developed differentiating techniques have suggested the existence of numerous other proteins with mRNA encoded in adipocytes and specific proteins formed and secreted from the adipocytes that achieve physiologically adequate plasma levels. For example, the protein designated Acrp30 is a novel serum protein similar to c1q, is produced exclusively in adipocytes, has plasma levels in ng/mL range, binds preferentially to muscle, has a cytokine tertiary structure and is regulated both acutely and chronically by insulin (34).

Modulation of risk factors for diseases, which are nutritionally regulated in fat tissue, may play a role in the longevity of the fat-depleted CR animals. We hypothesize that because evolution was typically associated with hunger and not with ad libitum feeding, the saturation of systems is easily achieved in human obesity and in ad libitum–fed animals. Fat depletion (by means of CR, pharmacologically or direct removal) has metabolic consequences on peripheral and hepatic insulin action, i.e., induces a reduction in peripheral and hepatic insulin resistance, an effect that seems to be independent of aging. In addition, although the accumulation of VF represents a risk for a variety of metabolic alterations (and manipulations at the level of VF may provide beneficial effects), it further proves the important role of fat in whole body homeostasis. In obesity, excess accumulation of FM may account not only for the perturbations in glucose metabolism, but also for a variety of additional risk factors for atherosclerotic cardiovascular and other unrelated diseases.

**LITERATURE CITED**