

# Caloric restriction, body fat and ageing in experimental models

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## Summary

Caloric restriction in animal models delays many age-related pathological conditions. Ageing rats have characteristically increased body weight, fat mass and a specific body fat distribution. This report will focus on the potential cause-effect relationship between increased fat mass and accelerated ageing. In humans, increased fat mass (obesity), and in particular increases in abdominal obesity as a result of deposition of visceral fat, are associated with the metabolic syndrome of ageing. This syndrome is associated with hyperinsulinaemia, dyslipidaemia, type 2 diabetes mellitus, atherosclerosis, hypercoagulability and hypertension. Fat tissue, however, plays a major role by secreting multiple metabolically active factors, which are potentially responsible for the development of insulin resistance. This article will review various experimental models (in animals) used to prevent insulin resistance of ageing by decreasing fat mass, and in particular, decreasing visceral fat. We suggest that this decrease in fat mass and its beneficial repercussions observed in ageing animal models may apply also to human ageing and its related pathology.

**Keywords:** Ageing, caloric restriction, insulin resistance, visceral fat.

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## Introduction

To live long is almost everyone's wish but to live well is the ambition of a few.

John Hughes

We begin this review with the human perspective, where age-related obesity is 'the Epidemic of 21st century'. Approximately 50% of the population in the US is overweight (1). In last few decades, with the advancement of medical science, the average life expectancy has increased but sedentary lifestyle and *ad libitum* (AL) intake of high-caloric refined food is the leading cause of obesity and its associated health hazards. In particular abdominal obesity, which is characterized by deposition of visceral fat (VF), is a prominent health hazard associated with risk factors for morbidity and mortality mainly from atherosclerotic diseases (2), cancer (3,4) and diabetes mellitus (5). Moreover, it has been demonstrated that intentional weight loss of up

to 19 lb by diet results in reduction in adjusted mortality by approximately 20% from all causes, by 40% from diabetes and by 10% from cardiovascular diseases (6,7). Thus leanness and obesity in humans may be similar to the paradigm of caloric restriction (CR) and AL feeding in animals and emerging evidence suggests that CR in obese people may increase longevity.

Ageing is often found to be associated with increase in fat mass (FM) and insulin resistance. In fact, the typical elderly person has normal body weight but increase in waist circumference, indicating abdominal obesity. People suffering from insulin resistance of ageing are found to have progressive increases in fasting and postprandial plasma insulin levels which are associated with increased incidence and prevalence of diabetes mellitus and impaired glucose tolerance (8–11). The association of generalized obesity and insulin resistance is well known. It is becoming increasingly apparent that beyond the effects of overall

obesity, the location of fat may have an additional impact in causing insulin resistance and other metabolic complications of obesity. Abdominal obesity in human is associated with increase in VF determined by computed tomography. Ageing is characterized by increase in VF relative to subcutaneous (SC) fat when compared to young people (12).

Increased FM and VF with ageing are associated with hepatic and peripheral insulin resistance (13–16). A large multi-centre study have demonstrated that age-related increase in FM determines the age-related decrease in peripheral insulin sensitivity (4,17), type 2 diabetes mellitus (18) and development of coronary artery disease (19), stroke (18) and death (20). Bjorntrop hypothesized that VF results in insulin resistance via ‘portal’ effect of free fatty acids (FFA) and glycerol released from increased omental fat (21). However, here we suggest that the observed relationship between increased FM and hepatic and peripheral insulin resistance may be caused by other endocrine and metabolic functions of VF.

It is evident that a complex relationship exists between ageing, obesity and increased VF, with the metabolic syndrome, insulin resistance and diseases associated with these conditions. To understand how these factors are interrelated and the pathophysiology of obesity, animal models play a crucial role. The advantage of an animal model is that dietary, drug or surgical interventions can be easily conducted in a homogenous group and subsequently followed up to analyse the physiological and molecular mechanisms (22). Using these models, effect of CR, VF and SC fat on insulin resistance and ageing can be studied separately. Several animal models have been described here and findings from these studies may explain the observations in human studies.

We reason that rodents used as experimental models are good paradigm to study the impact of sedentary lifestyle and AL food intake in human. Indeed, rodents in the wild are lean and are running approximately 10 miles a day while searching for food. However, rodents used in the lab with a restricted space to move and normally palatable food will cause them to have gain in weight and FM. Most importantly, restricting their food intake will extend their life dramatically (11). However, we would like to emphasize that no such studies have been performed in humans and the applicability of the results to the human paradigm (although obvious in some aspects) should be considered cautiously.

### **Caloric restriction and insulin action in rat model**

CR refers to a dietary regimen low in calories but nutritious. CR has got remarkable broad effects on increasing lifespan and attenuating chronic diseases of ageing in rodents (23). McCay’s pioneering work about 60 years ago

showed that CR increases lifespan by about 35% (24). Subsequently, CR in animal models has been used to explore different aspects of ageing (25).

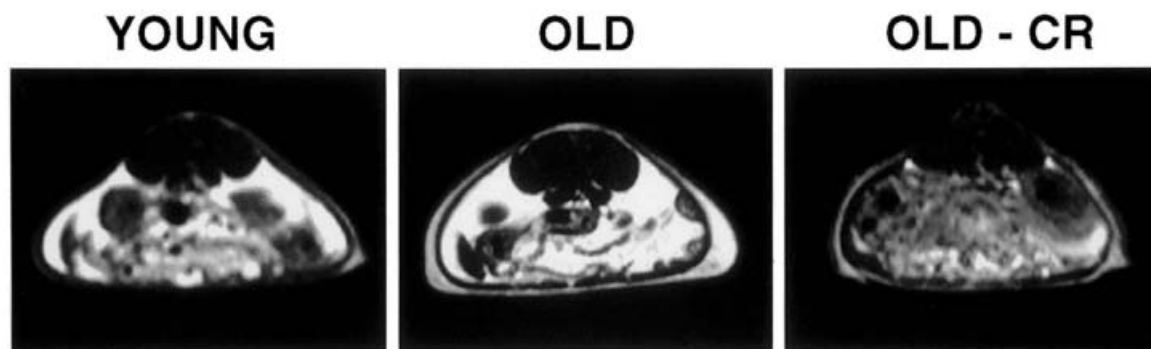
As in human, rodent develops increases in FM, VF and insulin resistance with ageing (26). Physiologically, insulin regulates glycogenolysis and gluconeogenesis in the liver, to control hepatic glucose production. Insulin also promotes glycogen synthesis in the muscle and triglyceride synthesis in the adipose tissues. Therefore, insulin resistance could be hepatic or peripheral (muscle and adipose tissue) or both. To examine the relationship between FM and hepatic insulin action with ageing, young, old AL and old CR Sprague-Dawley rats were studied using the Hepatic-Pancreatic clamp (26). Insulin was infused at a variable rate to chronically catheterized, awake and unstressed rats to clamp the blood glucose at a fixed basal level (26). There was a one-third decrease in the total FM and in VF in old caloric restricted rats compared to old AL fed rats. At the same time, these old CR rats were found to have their body fat redistributed (increase in total FM and a significant decrease in VF as compared to young AL rats (Fig. 1).

Thus in this study model, the effects of variable VF on hepatic insulin sensitivity could be assessed, independent of total FM and age. The insulin requirement to maintain euglycaemia was far greater in old AL followed by young AL and it was least in old CR rats. Interestingly, when these old CR rats were fed AL, VF increased rapidly and selectively associated with marked hepatic insulin resistance. This model supported the notion that VF plays an important role in determining hepatic insulin resistance, and CR effectively reverses hepatic insulin resistance to the level of young rats.

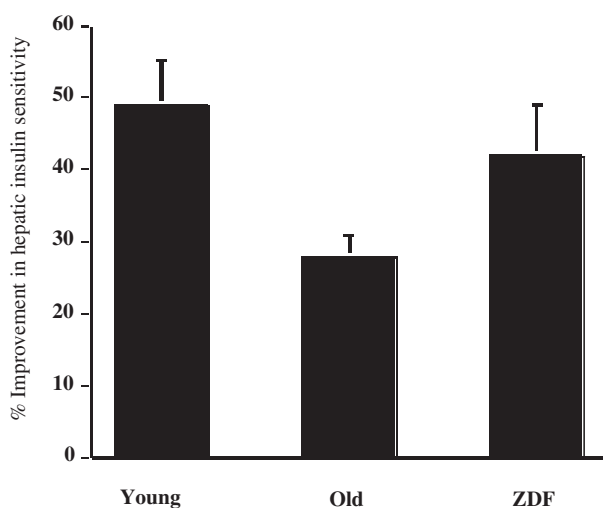
To determine the effect of FM and ageing on peripheral insulin sensitivity, a CR rat model was used (27). Young and old F1 hybrid of Brown Norway-Fischer 344 rats were fed AL and CR diet. During a physiological hyperinsulinaemic clamp (insulin was infused continuously and a variable amount of glucose was infused), glucose uptake rate (Rd) and glycogen synthesis rate (measure of peripheral insulin sensitivity) were significantly higher in young and old CR rats when compared to their AL counterparts. From this study, it was concluded that CR maintained peripheral insulin sensitivity at youthful level by preventing development of age-related development of FM.

### **Effect of visceral fat removal on glucose intolerance**

To study whether VF removal alters the natural history of glucose intolerance and diabetes, Zucker (+/+) rats were used as a model of ‘diabesity’ (diabetes mellitus associated with marked obesity) (28). Before the development of glucose intolerance at 2 months of age, these rats were assigned into two groups (1) VF– in which epididymal and



**Figure 1** Magnetic resonance imaging scans of the abdomen in young, old and caloric restricted (CR) rats. Cross-sectional cut above the level of the pelvis. The white colour depicts fat tissue. The old *ad libitum* fed rat has significantly more fat tissue (visceral and subcutaneous), compared to the young animal. By contrast, in old CR animal there was a marked reduction in both visceral and subcutaneous fat stores.



**Figure 2** Effect of visceral fat extraction on hepatic insulin action. Hepatic insulin sensitivity was assessed at physiological insulin levels during a clamp in young, old and Zucker diabetic rats (ZDF) that underwent epididymal and perinephric fat extraction or sham operation. In both young and ZDF groups, hepatic insulin sensitivity was significantly increased.

perinephric fat pads were removed and (2) VF+ receiving a sham operation. In this study, post-absorptive plasma glucose and FFA remained similar in both groups but fasting plasma insulin concentration decreased by approximately 50% in VF- group as compared to VF+ group. When using the pancreatic clamp (exogenous somatostatin and insulin infusion), and plasma insulin concentration was kept similar in both groups, glucose infusion was needed only in the VF- group to maintain plasma glucose at basal level, whereas VF+ became hyperglycaemic during this period. There was also a significant increase in plasma FFA concentration in VF+ group, suggesting resistance to the antilipolytic effect of insulin as well. Although plasma insulin concentration was similar, insulin-induced suppression

of endogenous glucose production (EGP) in VF- rats was comparable to those of the young rats (Fig. 2).

A follow-up study to examine the appearance of diabetes, measuring fasting plasma glucose concentration in the control ZDF rats demonstrated a significant delay in the development of diabetes in the VF- rats compared to the VF+ rats (Fig. 3). The development of diabetes was associated with the appearance of fat. In fact, as long as VF was maintained below approximately 40% of that of the controls, diabetes was prevented.

The reduction in VF by CR is the result of a proportional decrease in all VF depots, while in the surgical model it is entirely owing to decreased epididymal and perinephric fat. Thus, decreased VF could largely account for the beneficial metabolic effects of chronic CR. However, this study cannot quantify the relative contribution of mesenteric, epididymal and perinephric fat depots in mediating these effects.

### Effect of visceral fat removal on age-related insulin action

In order to evaluate whether VF play a causative role in peripheral and hepatic insulin resistance of ageing, F1 hybrid of F344/Brown Norway rat models were studied because of their extended lifespan (28). They were allocated into four different groups: VF- (VF extracted), CR (rats subjected to caloric restricted and sham operation), SC- (equivalent SC fat extracted), and young rats. All animals were analysed at baseline (Fig. 4a,b). During hyperinsulinaemic clamps (insulin with somatostatin infusion), VF extraction resulted in approximately 80% increase in rates of glucose infusion to maintain euglycaemia compared to SC fat-extracted group. The glucose uptake (Road) was significantly lower in SC fat-extracted group compared to the other three groups (young, VF- and CR group) (Fig. 4c). In this experiment, the action of insulin to suppress EGP was significantly affected by removal

of VF. VF- group demonstrated significant increase in the ability of insulin to suppress the glucose production compared to old SC- group. Insulin action in young rats and CR rats were similar as obtained in VF- group.

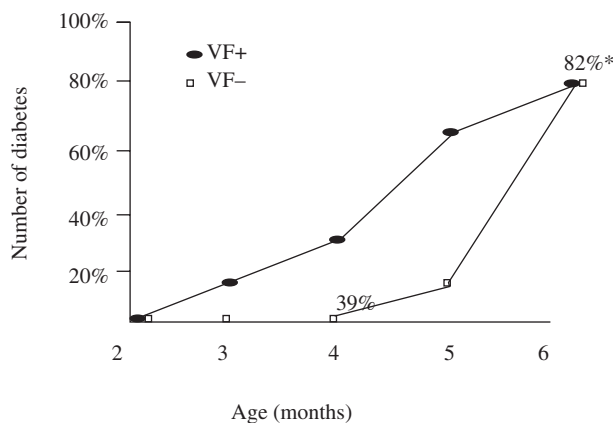
Of special note, insulin action improved in this model to the levels noted with CR. Thus, nutrient deficiency is not needed to obtain the improvement in insulin action in ageing rats. Taken together, this data supports a cause-effect relationship between visceral obesity and insulin action in young (27), old (28) and diabetic models (28) by surgically removing VF. The question that remains is what the mechanism by which VF induces insulin resistance is. While FFA was suggested as the mediator of hepatic insulin resistance through the increase in portal vein FFA level (29), our models had unchanged levels of venous and portal FFA levels. In addition, the perinephric and epididymal fat pads are drained by venous route, questioning whether portal FFA levels are needed for VF-induced insulin resistance. In the following sections, we suggest that fat-derived

peptides modulate insulin action, and by removal of VF the role of these peptides decreases significantly (23).

### Fat derived peptides and insulin resistance

Recent evidence indicates that adipose cells are also capable of biosynthesis and secretion of several metabolically active factors like leptin, tumour necrosis factor  $\alpha$  (TNF- $\alpha$ ) (30), resistin (31) and ACRP30 (32). These factors circulate in plasma and are active at distant tissues and organs. Some of these factors are responsible for the development for insulin resistance. Thus, surgical removal of selective fat pads may have removed an important factor implicated in the pathophysiology of insulin resistance and/or may have disrupted the cross talk between fat depots and potential distant targets (33).

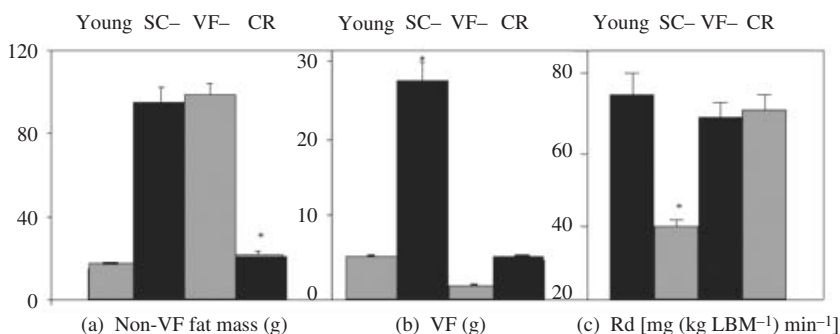
Some of these factors like leptin and adiponectin increase insulin sensitivity. *Adiponectin* (ACRP30) is a fat-derived peptide. It increases insulin's ability to suppress hepatic glucose production. It is also selectively expressed more in VF compared to SC fat (23,34). Other factors like TNF- $\alpha$  and resistin induce insulin resistance. *TNF- $\alpha$*  is a cytokine secreted from adipocytes and macrophage in acute and chronic inflammation and cancer. It mediates insulin resistance by decreasing insulin receptor tyrosine phosphorylation, insulin receptor substrate-1 phosphorylation, and down-regulating the mRNA for GLUT-4 (insulin responsive glucose transporter). It is expressed more in VF compared to SC fat (23). Resistin is a fat-derived peptide, expressed more in VF than SC fat. It mediates insulin resistance and its expression is decreased by insulin sensitizer rosiglitazone (31,34). However, its expression and role in humans has not been determined yet.



**Figure 3** This figure compares the frequency of diabetes with age in visceral fat (VF)-extracted (at 2 months of age) rats (VF-) and those with VF that have undergone a sham operation at 2 months of age (VF+). Half of the rats from each group were studied using a pancreatic clamp. The other half was followed up for 4 months until they developed diabetes (blood glucose more than 12 mmol L<sup>-1</sup>). The VF- rats studied at 4 months of age had 38% of the VF observed in VF+ rats. \*When diabetes appeared, VF in VF- rats accounted for 82% of that observed in VF+ rats.

### Leptin and modulation of body fat distribution

Leptin is a peptide expressed and secreted from adipose tissues. It acts through reduction in neuropeptide-Y expression in the hypothalamus (23) and have many others peripheral action, mainly through the melanocortin pathway (35). It decreases caloric intake and increases energy expenditure in human and rodents. It is expressed more in

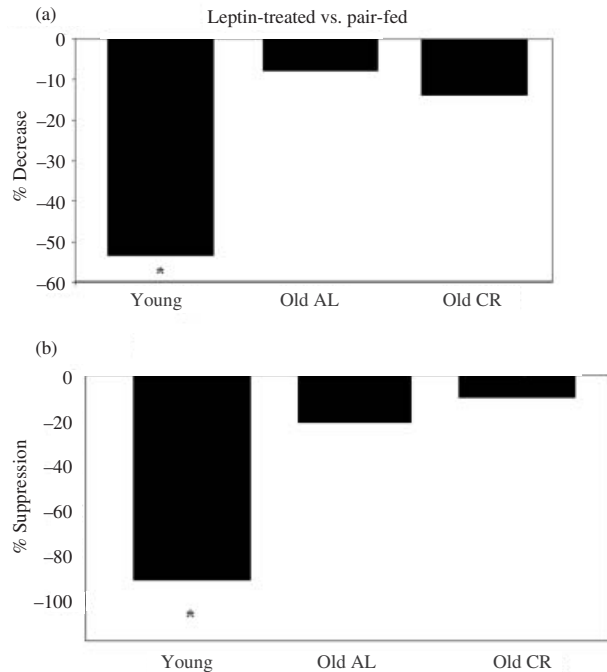


**Figure 4** Effect of visceral fat (VF) extraction on peripheral insulin action. Rats were allocated into four groups as young, subcutaneous fat extracted (SC-), VF (epididymal and perinephric fat) extracted (VF-), and caloric restricted (CR). These figures compared non-VF fat mass (a) and VF (b), and their glucose uptakes are compared in tissues in mg kg<sup>-1</sup> LBM min<sup>-1</sup> (c). LBM, lean body mass.

VF compared to SC fat (10,34). Obese individuals have marked elevation in plasma leptin directly proportional to increased FM (36,37). Conversely, leptin treatment to *ob/ob* (leptin deficient) mice leads to decreased food intake, body weight and FM through distinct hypothalamic pathways (36).

Leptin and insulin increases with age (38) but it is difficult to distinguish between the effects of obesity and that of ageing. In order to overcome this problem, we employed CR throughout ageing in a rodent model, and prevented the typical age-related changes in body composition and insulin action. To find out whether ageing and/or obesity are responsible for development of leptin resistance, rats were assigned into young (3 months) and old (20 months) groups and fed with either AL or CR diet (39). They were infused through intracerebroventricular route with either leptin or saline via implanted mini-osmotic pump and the infusion was maintained for 7 d. To demonstrate the effects of leptin on changes in fat distribution, independent of food intake, this study compared animals treated with leptin and pair-fed controls with matching body weight, per cent of total fat, and VF. It was found that CR rats irrespective of their age maintained somewhat higher physiological leptin levels than young control but AL rats had approximately 10-fold higher leptin levels than young or CR rats. Leptin infusion induced a decrease in body weight that was greater in young rats compared to old AL rats. Leptin administration failed to decrease the VF in the old rats, resulting in higher amount of VF, as compared to the young leptin-treated animals (Fig. 5a). Moreover, old CR rats were also resistant to leptin; its level was twofold higher in old AL compared to young. The action of insulin was also studied in these groups: it was found that EGP was decreased by approximately 90% in young rats treated with leptin compared with approximately 60% in the young pair-fed animals. Thus leptin augmented the insulin-induced decrease in EGP by approximately 30%. In contrast, the decrease in EGP in old animals with a similar degree of hyperinsulinaemia was approximately 50% in both leptin-treated and pair-fed animals (Fig. 5b). Thus, ageing significantly decreases leptin-induced insulin mediated suppression of EGP (36,39,40).

There are other important pathways that are also responsible for redistribution of body fat. Activation of peroxisome proliferator-activated receptor- $\gamma$  (PPAR- $\gamma$ , a nuclear receptor, selectively expressed in VF compared to SC fat) by pharmacological agents like thiazolidones can also decrease visceral adipose tissue while total FM remains unchanged (41). Body fat distribution can also be modulated in ageing humans by corticosteroids (increase VF) (42), growth hormones (43,44), insulin-like growth factors (IGF) (45,46), androgens (47) and oestrogens (48).



**Figure 5** Effect of chronic physiological increase in plasma leptin levels on visceral fat (VF; a), and the suppression of endogenous glucose production (EGP; b): Young and old *ad libitum* (AL) and old caloric restricted (CR) rats where implanted with mini-osmotic pump with either leptin or saline. (a) Represents the percentage decrease of total VF (i.e. perinephric, mesenteric and epididymal VF depots) by leptin in young and old animals compared with paired-feeding. The excess ability of insulin to suppress of EGP in the presence of leptin compared with pair-fed animals is presented in (b). EGP was approximately threefold higher in young pair-fed animals than in the leptin group, while leptin did not add to the ability to suppress EGP in old rats.

### Biological differences between visceral fat and subcutaneous fat

From the above discussion, it is evident that many of the fat-derived peptides are found to be associated with insulin action in several biological systems. Fat plays a significant role in modulating insulin action. It can be hypothesized that VF has different biological function from SC fat. In order to analyse this theory specifically, Atzmon *et al.* have examined the expression of genes in these two fat depots and quantified some of these differential expressions by real-time PCR (RT-PCR) methodology (49). Of the 1660 genes that were expressed in fat tissue, 297 (17.9%) genes showed a twofold or higher difference in their expression between the VF and SC fat (Table 1). Many of these genes are involved in glucose homeostasis and insulin action (PPAR- $\gamma$ , IGF1BP-3, IGF-1, GLUT-1), or in lipid metabolism (HMG CoA synthase, lysosomal acid lipase, hormone sensitive lipase). In other studies, TNF- $\alpha$  (30), resistin (31) and Acrp30 (32) genes were also shown to be expressed in significantly higher amounts in VF compared to SC fat. Recent studies demonstrate that the administration of

**Table 1** Quantitative changes in gene expression of GAPDH, leptin, PPAR, resistin and ACRP30 in visceral fat (VF) vs. subcutaneous (SC) fat. mRNA obtained from pairs of VF and SC adipose tissue from five young rats, analysed by quantitative real-time PCR (qRT-PCR) technology. *P*-values are between the expressions of the candidate genes by qRT-PCR in the two depots

Gene	Change VF/SC (fold) (qRT-PCR)	<i>P</i> -value (VF vs. SC)
GAPDH	1	NS
Leptin	0.8 ± 0.13	0.02
PPAR	4.13 ± 2.9	0.02
Resistin	12.2 ± 2.73	0.0001
Acrp30	3.8 ± 1.03	0.003

Acrp30 improves insulin action on hepatocytes and increases the ability of insulin to suppress glucose production *in vivo* (32). The markedly increased resistin expression in the VF of young rats and old diabetic rats (31) suggests a potential role for this peptide in VF-mediated insulin resistance. The gene expression of VF is markedly different from SC fat, which may account for the differences in the metabolic functions between the two fat depots. Taken together, these data provide an insight into the biological differences between VF and SC fat in rodents.

## Conclusion

In conclusion, in the study of the biology of ageing, CR extends life in a variety of species and decreases or delays the development of most age-related diseases. Most of the life-extending benefits of CR underlies its ability to reduce fat stores, especially the VF and their products. This process is regulated in particular by leptin and other peptides mediating their action through central nervous system (CNS) receptors. The reproducibility of this phenomenon makes its validity to be irrefutable. Although CR may increase life expectancy in abdominal obese individuals by delaying the development of cardiovascular disease and cardiac death, other life-extending effect of CR may be important. For example, low levels of IGF-1 characterize CR, and genetic and transgenic models of low IGF-1 live longer. Oxidative damage of macromolecules may be a key player in the ageing process and may be modulated by CR but not necessarily by extraction of VF. Finally, age-related diseases involving organs that do not seem to be directly associated with abdominal obesity may also be targeted by CR by other mechanisms. Future studies are designed to analyse whether manipulation of adipose tissue or various adipose-dependent signals may influence life expectancy.

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