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REVIEW

AN ADIPOBIOLOGICAL MODEL FOR WEIGHT REGAIN AFTER WEIGHT LOSS

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Abstract

Within one year, regain of a considerable amount of the weight lost by calorie restriction is a common phenomenon for up to 80% of individuals. This has been mainly attributed to psychosocial and behavioral influences. However, our recent findings point to a potential involvement of adipocytes in the risk for weight regain. During weight loss adipocytes build up resistance against releasing more fat, which is demonstrated by the differential expression of markers for cellular stress. This stress may be based on mechanical forces that arise between the shrinking cell and the surrounding rigid basal lamina. For adipocytes the best way to alleviate this stress is by returning to their original volume, which can be achieved by re-storage of triglycerides. To ascertain a sufficient supply of glucose and fat for re-storage, adipocytes change their pattern of secreted adipokines altering the total body metabolism and promoting energy intake. As a consequence the host will regain weight. Further research is needed to prove this model. If the model receives further support, the existence of an adipocytes-based autonomous cause for weight regain may be taken as an excuse to abandon measures needed to maintain the reduced weight. Therefore, care must be taken in the presentation of this model to the public. On the other hand, the model will also provide novel ways for prevention of weight regain.

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Introduction

Overweight and obesity are a worldwide threat to human health and is becoming a burden for national health care systems because of the associated disorders like cardiometabolic diseases, including atherosclerosis, hypertension, type 2 diabetes and the metabolic syndrome. Obviously there is an easy remedy because the risk for health complications can be reduced simply by losing weight (1). However, the real problem lies in the fact that up to 80% of people after losing weight regain a significant portion of the lost weight already within one year (2-6). Often this culminates in a cyclic pattern of weight loss and weight regain referred to as the 'yoyo-effect'. To stress the problem, members of the Netherlands Obesity Association stated that "obese people are most successful in losing weight. For that they often receive awards. But what they cannot achieve is to maintain their reduced weight". Obviously, knowledge about the factors influencing the risk for weight regain is of strategic importance to deal with the growing burden of obesity.

Here a model is presented that conceptualizes an adipocytes-driven influence on weight regain after weight loss.

The mainstream of weight regain research

A considerable number of studies has been undertaken to search for factors influencing weight maintenance after weight loss However, the results of those studies are difficult to generalize because they often differ with respect to the intervention program, use of calorie intake restriction with or without guided exercise program, length of weight loss period, the parameter to express body weight loss/regain (%BW or BMI, amplitude of BW, etc), extent of lost body weight (more or less than 10%), length of the follow up period, follow-up as controlled or non-controlled trial, and amount of body weight regain over time as cut-off for success of weight maintenance. Nevertheless, the outcome of such studies has been excellently reviewed in 2005 by Elfhag and Rössner (7). According to their analysis, weight maintenance is indicated by higher initial weight loss, motivation to lose weight, reaching a goal weight, a high level of physical activity, a regular meal rhythm, control of over-eating, self-monitoring of behavior, social support, ability to handle life stress, self efficacy, autonomy, taking responsibility in life, psychological strength and stability. Factors indicating risk for weight regain are: a history of weight cycling, disinhibited or binge eating, stress-eating, more intense feeling of hunger, difficulties in dealing with problems. Several more recent studies and meta-analyses have confirmed many of those findings and have also revealed some other influences promoting weight maintenance including (prolonged) subject-therapist contact (4,8) either direct or indirect like through the email (9), consistent self-weighing (10,11), dichotomous thinking (12), less time on watching television (13), and acupressure (4). Also ethnicity has been shown to be of influence (3), which may in part have a genetic basis. Recently, positive adherence to the weight loss diet was found to predict successful weight maintenance in pre-menopausal women that was partly explained by a lower energy intake and a higher physical activity (14). In an interesting study, Vogels *et al* (15) gave guidelines to subjects after the weight loss phase. These guidelines contained information about diet, about exercise, about both, or about other topics than diet and exercise. It was found that the guidelines were only sustaining the chance for successful weight maintenance when they were opposite to a subject's capability or preference. It suggests that people automatically apply the helpful measures that they know well or of which they are fond of, and need novel challenges to support them in maintaining weight. People who already regularly engage in physical exercise, are better of with guidelines on diet.

A long-term study among 4558 pre-menopausal women that intentionally had lost more than 5% of their body weight showed that 80% of them had regained more than 30% of the lost weight after 6 years. However, it was observed that weight regain was less in women who maintained moderate daily activity (16). The level of physical activity is a commonly recognized factor influencing weight regain. Increased body weight is associated in general with reduced physical activity and so is rebound weight gain, but at the same time the energy consumed in performing similar activities as before is increased (17,18). Reversely, when weight is lost, the energy spent on performing physical activity is considerably reduced (19, 20). In fact, for unclear reasons energy expenditure drops ~15% below the level that would be expected based on the lower body weight (21,22). This on itself can lead to a positive energy balance after weight loss that can be (partly) compensated for by an extra increase of the level of physical activity. Failure to maintain such level, will add to the risk of weight regain. It has also been reported that if the decrease in energy expenditure is not accompanied by a sufficient reduction in energy intake, this will increase the risk for weight regain (23).

Altogether it shows that the mainstream of explanatory research for weight regain after weight loss has focused on psychosocial and behavioral influences. Biological responses of the body to dieting with influence on weight regain, as mainly studied in rodents, have been reported as well (24). Here I present another biological influence on weight regain merely based on the behavior of the adipocytes in the body.

Adipocyte resistance against release of fat

Loss of fat seems most easy in the beginning of a weight loss intervention, but gradually becomes less. In a study by Diepvens *et al* (25) subjects lost 4.2 kg of body weight during 8 weeks on a low calorie diet. However, it was observed that in the second four weeks 3x less weight was lost (Fig. 1). It suggests that during the intervention the fat mass approaches an equilibrium level after which it becomes more and more difficult to lose additional weight. Löfgren *et al* (26) have shown that women who either by lifestyle modification or bariatric surgery went from the obese to the non-obese state in about three years, had a reduction in adipocyte cell volume of 43%. This indicates that the equilibrium may be reached when adipocytes have lost about 45% of their fat content. In a 12 month weight loss intervention comparing different diets, the leveling-off of weight loss over time was clearly demonstrated (27). *In vitro*, when mature mouse 3T3-L1 adipocytes are cultured with minimal glucose in the growth medium, they also readily lose about 46% of their fat content as measured by oro-staining (28). Only in the presence of tumor necrosis factor-alpha (TNF-α) fat release is faster and reaches 77%.

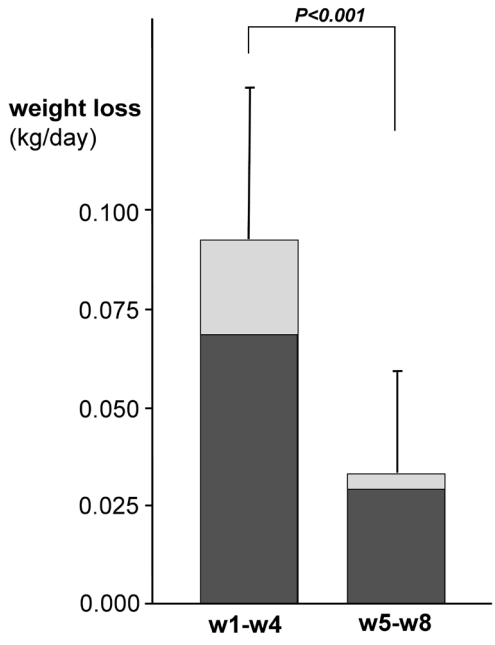

Figure 1. Weight loss over time. Results from an 8 week low calorie diet intervention on the loss of body weight and the loss of fat mass (dark bars), in which 46 women with a BMI of 27.7 $\pm$ 1.8 kg/m2 participated (data from (25).

What those observations suggest, is that some kind of resistance against fat release builds up in adipocytes in parallel to the loss of fat. In evolutionary terms such a restrained release of fat may make sense, because part of the stored fat might be used to survive a period of famine providing fuel for the body's cells including the adipocytes, while sufficient energy would be kept stored as fat for facing a possible second hit on the undernourished organism, for instance an attack by pathogens. Indeed, addition of the inflammatory marker TNF-α to low glucose-treated 3T3-L1 adipocytes seems to facilitate the release of fat (28).

An ECM-related mechanism for adipocyte cellular stress

A question then is what the nature of the cellular stress could be. Studying protein turnover in 3T3-L1 adipocytes by proteomics analysis of stable isotope labeled proteins, we found that mature adipocytes spend a large part of their metabolic energy on the rapid turnover of collagens, whereas the turnover of proteins from the cytoskeleton was considerably slower (29). What it shows is that mature adipocytes put a lot of their energy into the maintenance and dynamics of the extracellular matrix (ECM). Insulin is an important promoter of ECM formation of adipocytes, although not by transcriptional up-regulation of genes for ECM-proteins but rather by the up-regulation of the genes involved in processing and maturation of ECM-proteins (30). In fact, in the adipose tissue the adipocytes are surrounded by a relatively thick ECM referred to as basal lamina (31). Adipocytes share this characteristic with their lineage members: chondrocytes and osteocytes in cartilage and bone. Mature human adipocytes are mainly composed of a single droplet of triglycerides surrounded by a phospholipid monolayer, which is vulnerable to breakage. The purpose of the strong ECM is therefore to protect the adipocytes from mechanical disruption. Recently, a role of the ECM in adipocyte hypertrophy, formation of fibrosis and the transition of the tissue to a state of chronic inflammation has been proposed (32). Based on the essential function of the ECM for adipocyte maturation and survival, it is tempting to propose that the ECM also has a relevant role in weight regain after weight loss. Microarray gene expression analysis of adipose tissue RNA has shown repeatedly that genes for ECM-proteins are differentially expressed during weight loss (33,34). Recently, Mutch *et al* (35) investigated gene expression in subjects from the pan-European Diogenes study, which involved at least 8% weight loss in two months with a weight maintenance follow-up of 6 months. When at the end of the intervention gene expression of adipose tissue was compared between subjects that regained only 0-10% (WM, weight maintainers) and those who regained 50-100% (WR, weight regainers) of the lost weight, genes of the focal adhesion pathway that play an important role in the coordination of the ECM, were seen to be down-regulated in WMs while up-regulated in WRs. It underscores the importance of the ECM of adipocytes in weight regulation after weight loss.

When overweight/obese people lose weight, a larger part of the lost weight is constituted by fat that is released from adipocytes. As a consequence, adipocytes will shrink. If on average the mature adipocyte volume is composed of 85% fat and 15% non-fat cell material, a reduction of 45% of the fat will result in about 60% reduction of the overall volume. Assuming a spherical shape, the diameter of the cell will reduce by about 15% and the surface by about 72%. Apparently, at that stage something may prevent the further release of fat. If cells shrink, their surrounding ECM has to be adjusted as well. However, remodeling of the ECM involves the turnover of ECM proteins like collagens. For the construction of new collagen fibers, collagen proteins have to be synthesized and modified, for instance by prolyl-hydroxylase, which is an energy-demanding process (31). However, under conditions of calorie restriction during weight loss, such energy is not available. As a consequence, the adaptation of the ECM cannot keep up with that of the cell and stress will build up between the ECM and the cell due to traction forces inducing a cut down on the further release of fat. That the rigidity of the ECM can indeed influence signaling processes in the adipocytes has been shown by *in vitro* experiments (36).

Searching for indications that adipocytes after weight loss display signs of cellular stress, we used proteomics to study the protein dynamics in adipocytes during weight loss by a very low caloric diet (37). Several of the identified proteins were found to belong to the so-called minimal stress proteome and are present on the list of generally detected proteins (38, 39). Of those, the proteins involved in cell metabolic processes had relatively low fold changes during weight loss (ATP synthase beta (-1.25), GAPDH (-1.15), ALDOA (1.23)), whereas those with the highest fold changes were tubulin B5 (1.86), and an isoform of vimentin (-2.22) and ALDOC (-2.62). Although aldolase may be regarded as a metabolic enzyme involved in glucose conversion, in the past it has been found to be a component of the cytoskeleton involved in the scavenging of Glut4-containing particles (40). It seems therefore, that stress proteins in adipocytes with the highest response to weight loss are less related to metabolism but more related to cell structure (Fig. 2). It indicates that the cells suffer greatly from structural stress as would be expected in the case of traction forces.

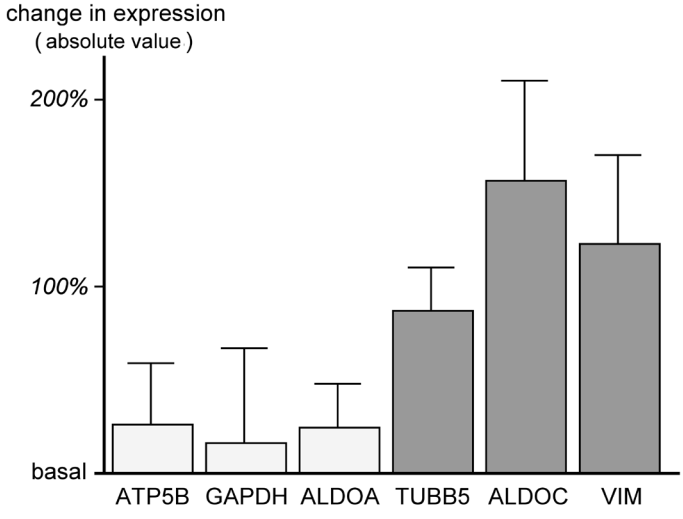

Figure 2. Changes in expression levels of stress proteins. Proteomics of adipocytes before and after a very low caloric diet for 5 weeks followed by 3 weeks of a balanced diet showed changes in the expression of various proteins belonging to the minimal stress proteome. Changes in expression (absolute values; error bars indicate SD; n=8) are shown for proteins related to cell metabolism in light gray and for those related to cell structure in dark gray. ATP5B (25 $\pm$ 32): ATP synthase subunit beta; GAPDH (15 $\pm$ 47): glyceraldehyde-3-phosphate dehydrogenase; ALDOA (23 $\pm$ 23) fructose-bisphosphate aldolase A; TUBB5 (86 $\pm$ 23): tubulin beta chain; ALDOC (162 $\pm$ 55): fructose bisphosphate aldolase C; VIM (122 $\pm$ 47): vimentin.

The consequences of adipocyte cellular stress

The cellular stress that accumulates in adipocytes when people lose weight due to the discrepancy between the shrinking of the cell and the ECM can theoretically have a different follow-up. First, cells might decide to go into apoptosis. However, the relatively long average lifespan of 10 years for adipocytes suggest that apoptosis is not a likely choice of adipocytes (41). Apoptosis would lead to a high turnover of adipocytes and accumulation of fibrotic material during weight loss, which has not been reported. Secondly, the stress may gradually fade as the ECM adjusts to the reduced cell volume. As already mentioned, under conditions of energy restriction this would be a slow process. Even after return to a state of energy balance it could be a considerable undertaking for each cell to reconstruct its basal lamina, which could actually mean a reconstruction of the entire tissue. In this case, part of the created tissue volume might be filled by differentiation of preadipocytes leading to an increase in the number of adipocytes in the tissue. However, Spalding *et al.* have reported that the number of adipocytes in adults does not change much (42,43). Yet, the fact that three years after bariatric surgery adipocytes have remained at a 40% reduced volume indicates that a gradual adaptation of the ECM over the years is possible (26).

Probably the efficient and low cost way for the adipocytes to get rid of cellular stress is to return to their original volume by storing fat again (Fig. 3) (44). The adipocytes even have special tools available that can be used to speed up such a process: adipokines. Under normal conditions they secrete numerous (poly) peptide hormones that regulate the metabolic activity of other peripheral tissues but also influence the energy intake (45). For instance, by changing the adipokine profile, adipocytes can manipulate the eating behavior of their host. In fact, Skurk *et al* showed that the secretion profile is related to adipocyte size (46) suggesting that during weight loss and shrinkage of cells, adipocytes will automatically change their adipokine secretion profile.

Leptin is one of those hormones that play a role in signaling the fat content of the body to the brain. Plasma leptin levels are associated to the amount of fat mass (47). If the stored fat content is sufficient, it is signaled by leptin to the brain and as a consequence appetite is repressed. The production of leptin is related to adipocytes size (46). Consequently during weight loss leptin levels decrease allowing the feeling of hunger. Remarkably, it has been repeatedly observed that during weight loss leptin levels drop far beyond what would be expected from the amount of lost fat mass. Typically, a 15-20% loss of fat mass is accompanied by a reduction of 40-60% in leptin (37,48). According to some researchers this leads to a state of leptin deficiency in the brain (49). For the adipocytes this is favorable because it would induce energy intake by the host supplying sufficient glucose and fat for storage of triglycerides and return to the original volume with relieve from cell stress. In addition to leptin, also other

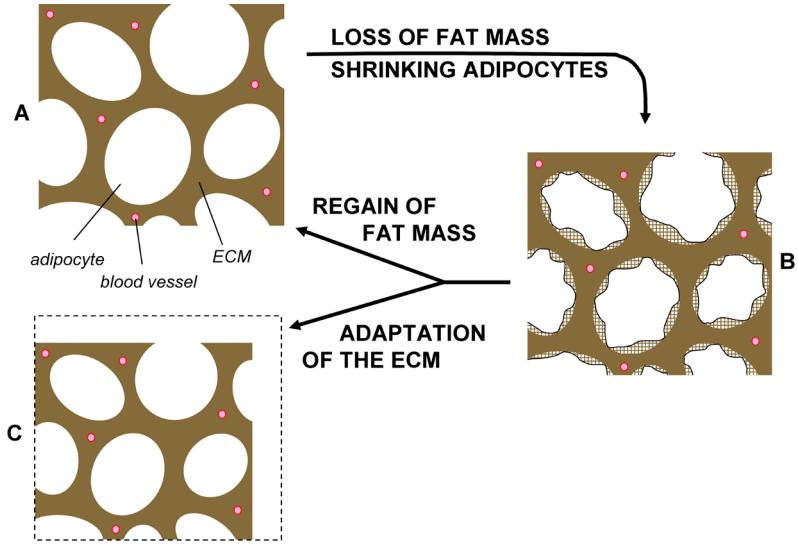

Figure 3. An adipobiological model for weight regain. During weight loss intervention, mature adipocytes embedded in their strong extracellular matrix (**A**) will lose fat and will therefore shrink (**B**). In the next phase adipocytes can return to their original state by re-storing fat (**A**). Alternatively, the cell surroundings might adjust which would result in adipose tissue with smaller cells (**C**). Under conditions of low energy supply to the body, this adaptation would be slow and return to the original state more likely.

adipokines may help promote energy flux towards the stressed adipocytes. For the host this would mean that after weight loss there is increased risk for weight regain originating from the cellular stress of the adipocytes.

Conclusion

In summary, in addition to psychosocial and behavioral influences, here a biological model for weight regain after weight loss is presented based on the behavior of adipocytes, which accumulate structural stress upon fat release that is most easily diminished by re-storage of fat (Fig. 4). For this, the adipocytes adjust their adipokine secretion increasing the energy-intake of their host in order to sufficiently supply triglycerides for storage. For the host this means renewed weight gain. The model is inspired by experimental results as outlined above, but needs further proof. If such proof can be provided, it will add an extra dimension to our understanding of the high incidence of weight regain after weight loss. Intrinsic factors originating from within our own body will be added to the causes of weight regain. Weight maintenance after weight loss is for most people difficult to achieve. At present failure to maintain a reduced weight may lead to feelings of frustration and self-blaming. With an intrinsic cause added to explain weight regain such feelings can be prevented, because the accumulation of adipocyte stress and

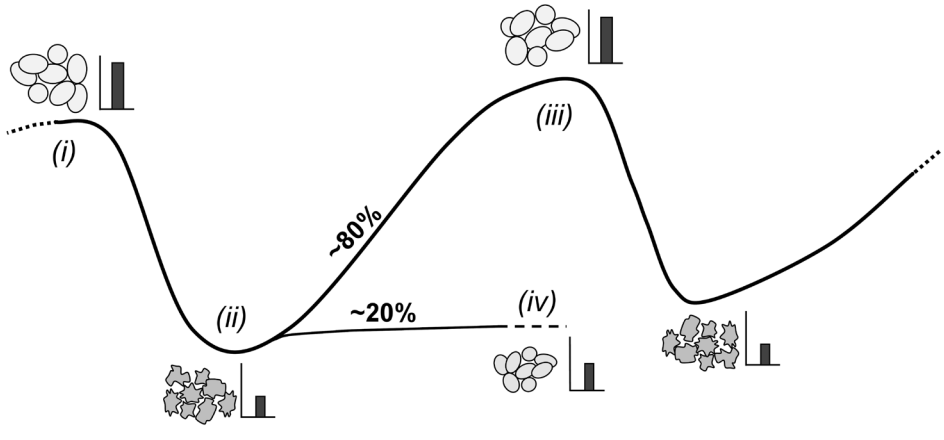

Figure 4. Adipobiology of weight cycling. The weight cycling is shown by the solid line. Obese people have large adipocytes and high plasma levels of leptin as shown by the bar (***i***). Upon weight loss adipocytes shrink and accumulate cell stress. Leptin levels are reduced by 40-60% (***ii***). The changed adipokine profile induces eating behavior and up to 80% of people return to the original weight or go beyond it. Leptin levels rise again dramatically (***iii***). Twenty percentage of people or more succeed in maintaining a lower weight with a reduced leptin level (***iv***). The others start on their next round of weight loss and attempt of weight maintenance (***iii***).

their subsequent reaction is autonomous and beyond our will. On the other hand, an adipocyte-based cause for weight regain might be taken by some as an excuse to easily abandon all attempts for weight maintenance. Therefore, care should be taken how the results from investigating this model will be presented to the public. Anyway, showing the contribution of adipobiology to the risk for weight regain can lead to new methods improving weight maintenance after weight loss by preventing adipocyte cellular stress or its consequences. In this regard, preventing the leptin drop has already been studied to some extend (50). Losing weight in a slow and controlled manner is thought to generate less stress than crash dieting. If true, this will also be of great help, but all those aspects of weight maintenance management have to be further studied.

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