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Mechanisms in Adipose Tissue: Does Fat Have Anything to Say About Lean Body Mass?

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Abstract

Obesity is associated with many adverse conditions. Adipose tissue contributes significantly to these conditions. Maintaining lean body mass is essential not only to the success of decreasing adipose mass but also to the maintenance of a healthy weight. We have investigated common cellular mechanisms in adipose tissue associated with lean body mass phenotypes induced by a high-protein diet, a diet containing conjugated linoleic acid, or exercise. Mechanisms include a decrease in inflammation, a decrease in fatty acid metabolic processes, as well as growth and development changes. These mechanisms and associated benefits stress the importance of maintaining lean body mass in animals.

Glossary of Abbreviations
CLA: Conjugated Linoleic Acid
Exe: Exercise
HP: High-Protein
IL-1β: Interleukin-1β
IL-6: Interleukin-6
LBM: Lean Body Mass
MCP-1: Monocyte Chemoattractant Protein 1
REE: Resting Energy Expenditure
TNF-α: Tumor Necrosis Factor-α

Obesity and Lean Body Mass

Obesity has become a major health problem in Western civilizations as well as in developing countries worldwide. There now may be as many obese individuals in the world as those suffering from hunger. Over 72 million people are obese in the United States alone.[1] There are a multitude of health issues and chronic diseases associated with obesity. These include diabetes, hypertension, coronary heart disease, certain cancers, osteoarthritis, as well as many others. In addition to humans, the obesity epidemic also affects pets. At least one-third of dogs and cats are overweight or obese.[2,3]

Obesity is characterized by the excessive storage of body fat due to an imbalance between caloric intake and energy expenditure tipped in favor of intake. Addressing obesity by decreasing caloric intake is obviously an important part of successful weight management, but it only represents one-half of the equation. Maintaining or increasing energy expenditure represents the other half. Lean body mass (LBM) is defined as the mass of the body minus the fat. This includes bones, muscle, skin and organs. LBM makes significant contributions to the energy expenditure. Therefore, maintaining LBM plays a critical role in the prevention of obesity and maintenance of a healthy weight.

In addition to serving as a reservoir for amino acids for endogenous protein synthesis and hepatic gluconeogenesis,[4,5] muscle is a major player in the expenditure of energy. Total energy expenditure includes the thermic effect of food, energy related to exercise or activity, and resting energy expenditure (REE). Of these, REE is the largest component.[6] Multiple tissues contribute to REE; however, muscle is the single largest contributor and the tissue that has the greatest ability to vary in energy expenditure.[5] Protein turnover, which is the synthesis and breakdown of endogenous proteins, uses between 20% and 33% of REE and is the principle contribution of resting muscle to REE.[5,7] A 5 kg difference in muscle roughly equals 50 kcal/day of energy expenditure for protein synthesis. This energy, stored as fat, would equal approximately 2.5 kg of fat mass per year.[5] This estimate may be low since the energy cost of protein breakdown was not considered in this estimate and requires additional energy.

Key Words
Adipose Tissue
Conjugated Linoleic Acid
Exercise
Gene Expression
High-Protein
Inflammation
Lean Body Mass
Microarray
Obesity

High-Protein Diets

One of the most common methods of maintaining LBM during weight loss is a high-protein diet. This has been well-documented in multiple species, including humans, dogs and cats.[8-13] In addition, REE is increased and the weight regain after loss decreased in subjects fed a high-protein diet.[9,14] Energy intake to maintain the rate of weight loss was lower and energy consumption to maintain a constant body weight after weight loss was

higher in cats fed a high-protein diet as compared to control.[14] Additionally, an increase in heat production has been shown in lean cats fed a high-protein diet during weight loss.[15]

The Role of Adipose Tissue in Obesity

While the bottom line of obesity is represented simply by more energy being stored as compared to energy being spent, the tissues, cells, molecules and systems involved are anything but simple. The process of body weight maintenance is a complex homeostatic system that involves both genetic and environmental factors. It involves multiple tissues and cell types, including brain, specifically the hypothalamus, gut, liver, pancreas, muscle, and adipocytes from various types of fat depots, immune cells such as macrophages, and many others. Additionally, these tissues and cell types are linked via hormones, cytokines, chemokines and other signaling molecules. Within each of these cell types are biological pathways represented by metabolic pathways, signaling pathways, genes and other molecular mechanisms.

Adipose tissue is now recognized as a dynamic tissue involved in autocrine, paracrine and endocrine signaling associated with many, if not all, tissues involved in obesity. Adipose tissue is comprised of multiple cell types that communicate among themselves through many of these same mechanisms. Adipose cells include adipocytes, preadipocytes, endothelial cells, blood cells, macrophages, as well as others. Signaling mechanisms include the production and expression of receptors for adipokines, cytokines, hormones, growth factors, etc., which allows for crosstalk between the cells contained in adipose tissue.[16]

There are a multitude of adverse effects associated with adipose tissue during obesity. These effects include inflammation, oxidative stress, macrophage infiltration, hypoxia, necrosis and a decrease in insulin sensitivity. However, inflammation plays a role in all these. Inflammatory responses in adipose tissue involves the abnormal production of proinflammatory cytokines, including tumor necrosis factor-α (TNF-α), interleukin-6 (IL-6), interleukin-1β (IL-1β), chemokines such as monocyte chemoattractant protein 1 (MCP-1), hormones such as adiponectin (decreases with obesity), among others. In addition to its proinflammatory activities, TNF-α has been shown to decrease insulin sensitivity.[17]

Adiponectin production is also involved, although a decrease in production contributes to a decrease in insulin sensitivity.[16,18] MCP-1 is produced by adipocytes and may be responsible for the recruitment of macrophages into adipose tissue.[19,20] Macrophages are a major source for the production of both TNF-α and IL-6.[19,21] Macrophage infiltration is high in obese, but not lean, individuals and is usually localized around necrotic adipocytes.[22] Local hypoxia may be a major cause of adipocyte cell death and has also been associated with the inhibition of insulin action in adipocytes.[23] All these adverse obesity-associated events have detrimental effects on adipose tissue and also contribute to more general disorders such as high blood pressure, coronary heart disease, diabetes, etc.

The Role of Adipose Tissue in the Lean Body Mass Phenotype

Inherently the induction of a LBM phenotype involves an increase in the ratio of lean to fat. This is normally obtained via a decrease in the mass of body fat with an increase, or maintenance, of muscle mass. Understanding the mechanisms associated with this phenotype in involved tissues, including adipose, can provide valuable insight into the underlying cellular and molecular processes involved in weight management, as well as provide novel targets for future studies. We previously investigated the mechanisms associated with the induction of a LBM phenotype in adipose tissue.[24] The goal of this

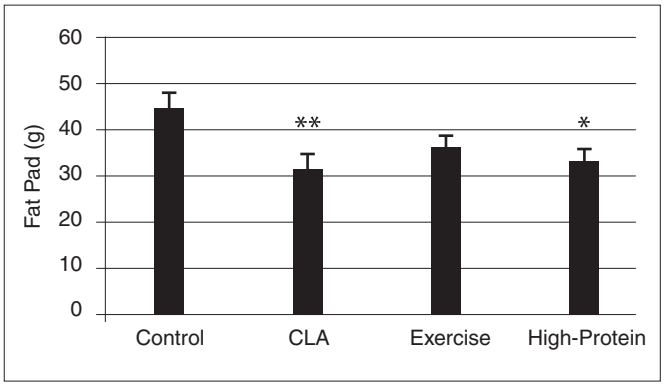

Figure 1. Fat pad weight of rats on control diet, conjugated linoleic acid (CLA) diet, exercise, and high-protein diet. Values represent the mean (g) ± SEM. * = P<0.05 and ** = P<0.01 as compared to the control.

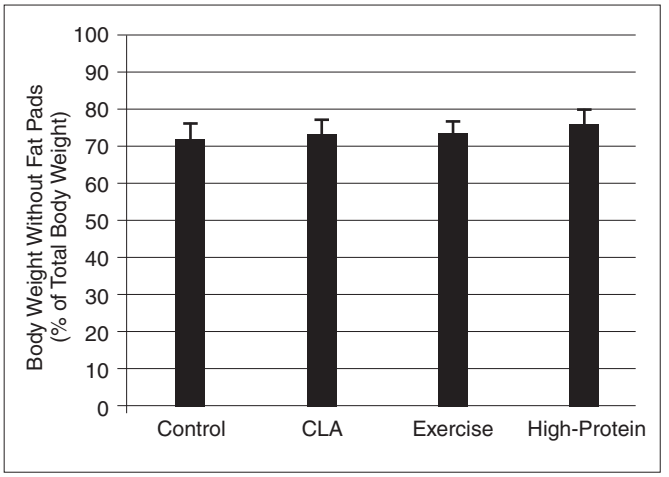

Figure 2. Body weight without fat pads (% of total body weight) of rats on control diet, conjugated linoleic acid (CLA) diet, exercise, and high-protein diet. Values represent the mean percentage ± SEM.

work was not to evaluate weight changes but to elucidate the mechanisms of and metabolic responses to maintaining (or increasing) LBM while decreasing fat. In order to differentiate common mechanisms associated with this phenotype from individual treatment effects, rats were put on three different LBM-enhancing treatments. The overlap of the molecular responses to those treatments served as the focus of this study.

Exercise, a high-protein diet and a diet containing conjugated linoleic acid (CLA) were used independently to induce LBM phenotypes. Exercise was used due to its ability to increase fat oxidation and modulate muscle metabolism. High-protein was used due to its ability to increase protein turnover, fat oxidation[25] and thermogenesis.[26] CLA was used due to its ability to increase body protein and reduce body fat.[27,28]

LBM phenotypes were validated by a significant reduction in fat pad weight as compared to control in the high-protein and CLA groups with a marginal reduction in response to exercise (Figure 1). Fasting blood glucose followed a similar trend with significant reductions in the high-protein and CLA groups with a marginal reduction in the exercise group. Insulin levels were directionally similar without significance.

The treatments had no significant effects on body weight, food intake or weight gain. Additionally, there was no significant increase in body weight without fat pads (percentage of total weight) representing the sparing effects of the treatments on LBM (Figure 2).

In order to elucidate the molecular mechanisms associated with LBM phenotypes, gene expression changes were assessed in adipose tissue samples using microarray technology. Gene expression is the process of transcribing DNA (a copy of a gene) into a messenger form (messenger RNA) and translating that message into its protein product. Alterations in gene expression occur in response to its environment, needs, etc. While the activity of proteins can be regulated at different levels (e.g., phosphorylation), gene expression is the main regulation step in overall activity of the protein.

The microarray used in our study contained over 30,000 different messenger RNAs. The overlap of changes, common to all three treatments, identified 784 significantly different gene expression changes as compared to control (Figure 3). These genes, and their protein products, were associated with three major areas of physiology. They are inflammation, metabolism and growth/development.

Inflammatory gene expression changes were mostly downregulated, implying a less inflammatory state in the LBM phenotype. One particular pathway within inflammation was leukocyte transendothelial migration. Macrophage infiltration is common in the adipose tissue of obese individuals.[19] However, we observed a down-

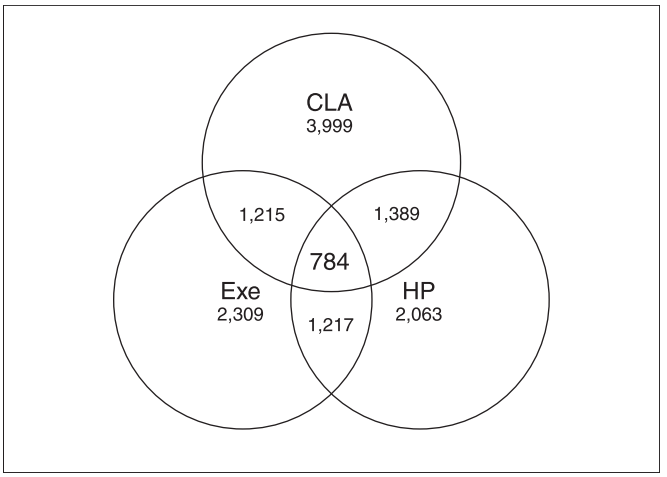

Figure 3. Gene expression changes in adipose tissue from rats on conjugated linoleic acid (CLA) diet, exercise (Exe) and high-protein (HP) diet as compared to control diet. Numbers under treatments represent total number of differentially expressed genes for that treatment compared to control, while other numbers represent genes differentially expressed in common among multiple treatments. Genes were determined as differentially expressed based on $P<0.01$ for overall treatment effect and $P<0.01$ for the specific treatment versus control.

regulation of this pathway even though our control animals were not obese, indicating that LBM phenotype can result in less inflammation partially due to a reduction in macrophage infiltration.

Metabolic changes included many enzymes (many rate-limiting) involved in fatty acid, lipid and cholesterol metabolism. Most of these enzymes were downregulated. Fatty acid metabolic changes included rate-limiting enzymes specifically involved in fatty acid synthesis, fatty acid breakdown and fatty acid elongation. This less active state is most likely due to the overall decrease in fat mass. The rate-limiting enzyme in cholesterol metabolism, HMG-CoA reductase, was also downregulated. This enzyme is the target of the cholesterol lowering drugs, statins. In addition to their cholesterol-lowering ability, statins are known for their anti-inflammatory effects.[29] The decrease in expression of HMG-CoA reductase in our study may indicate this as one of the major players in the decrease in inflammation associated with the lean body mass phenotype.

Growth and development changes included mechanisms associated with apoptosis, adipogenesis, as well as others. Most of these were downregulated in response to the LBM phenotype. Apoptosis has previously been shown to be associated with obese conditions,[22,30] and the decrease in fat mass may be reflected here. A decrease in adipogenesis may reflect the decrease in need, or ability, to replace fat cells. Since this study was performed with adult animals, adipogenesis would only represent the replacement of existing cells since the majority of new adipocytes are produced shortly after birth.[31,32]

Conclusion

Obesity is associated with increased risk of many chronic diseases that are detrimental to the health and longevity of animals. Underlying these disorders are various changes that contribute to the pathologies of the disorders. Adipose tissue is a major player in these pathological changes including inflammatory-related events that can result in a decrease in insulin sensitivity, hypoxia, macrophage infiltration and cell death. Decreasing adipose tissue mass is critical to reversing these pathological changes. However, the maintenance of LBM is also important. Preservation of LBM is not only critical to the success of weight management but also to the success of preventing weight regain as has been shown with multiple species using high-protein diets. In addition, preserving LBM during weight loss of obese animals and maintenance of LBM in non-obese animals results in favorable molecular events in adipose tissue, especially a decrease in inflammation.

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