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NUTRIPROTEOMICS FOR OBESITY RESEARCH

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ABSTRACT

This study aimed to review findings of scientific articles which the use of proteomic tools made it possible to characterize the metabolic profile of obese individuals. We searched the electronic databases: Scielo, PubMed, Lilacs and capes. The time period was defined as 10 years, having as main keywords: proteomics, proteome, obesity, biomarkers. The use of serum proteomic techniques proposed prognostic markers for diseases and diseases resulting from obesity, such as diabetes and cardiovascular diseases. An analysis can be performed from hepatic tissue, muscle, adipose tissue and body fluids such as saliva, urine, serum and plasma. In the obesity treatment, monitoring the effects of physical exercise may reveal which proteins can modulate this physical activity, thus contributing to a deeper understanding of the physiological mechanisms. Although caloric restriction for weight loss is the main intervention for treatment, other interventions aimed at reducing inflammation in adipose tissue have been studied. Prospective analysis of representative and well-characterized populations to obtain statistical power is a limitation of proteomic studies. Therefore, to increase the relevance of studies in nutriproteomics, in particular studies on obesity, will certainly have to focus even more on application in humans.

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INTRODUCTION

Obesity is considered as an excess of body fat that causes risk of non-transmissible diseases and early mortality. (WHO 2016) The projection for 2015 is 2.3 billion of overweight adults, including 700 million of obese, indicating a 75% increase in obesity cases over 10 years. Obesity accounts for 7% of global health costs. (Kitzinger and Karle, 2013) Among the factors related to the development of obesity, we can highlight the ingestion of hypercaloric diets, the inadequate consumption of fats and carbohydrates in the diet, absence of physical activity and genetic susceptibility that result in fat accumulation. This amount of stored fat is concentrated in the adipocytes, which increase both in size and number. (Lee *et al*., 2013) Adipose tissue is actively and strongly associated with metabolic disorders and a deeper understanding of the biological mechanisms that regulate its functions will help in the development of new strategies for obesity treatment. In this

context, proteomics appears as an important tool in the identification of the proteins that are related to obesity, allowing to evaluate the molecular mechanisms related to its genesis and its development. (Kim *et al*., 2015) Thus, this work aimed to review the findings of scientific articles where the use of proteomics tools in the nutrition study made it possible to characterize the metabolic profile of obese individuals.

MATERIALS AND METHODS

The present literature review was carried out by searching the electronic bases of scientific journals, such as: Scielo, PubMed, Lilacs and Periodical Capes. The time period was delimited in 10 years (2005-2015) and the following keywords were used in the search tools: proteomics, proteome, obesity, biomarkers, diet. In the search for papers that evaluated specific tissues, the key word proteomics and Boolean operator "AND" were used, followed by the name of the tissue of interest: liver or skeletal muscle.

Literature review

From the research performed here, 19 articles were obtained whose main findings are summarized below (Table 1). The material obtained reveals heterogeneity of experimental designs and analytical methodologies, which led us to group them into four axes: tissue proteome; proteome from body biofluids; effects of calorie restriction and physical exercise on obesity; and dietary treatments and propensity to obesity. At first, such methodological diversity could sound to the reader as an indication of the insertion of elements of confusion or even of low inference power. However, we emphasize here that the changes that constitute the metabolic signature of the obese individual were common, regardless of the methodology adopted, thus demonstrating the validity of the data presented here. Among these alterations, we highlight the impairment of energetic metabolism and antioxidant systems, as well as the presence of chronic inflammation and stress markers in the endoplasmic reticulum and mitochondria.

Proteom of tissues

Liver tissue

Using 2D-DIGE and MALDI-TOF-TOF, Rodriguez analyzed liver biopsies of morbidly obese patients at different stages of steatosis. (Rodriguez-Suarez *et al*., 2010) The authors found a significant down-regulation of CSP-1 and GRP78 proteins in patients with steatohepatitis, in relation to patients without hepatic alteration or change without the presence of inflammation, indicative of metabolic dysfunction and stress in the endoplasmic reticulum secondary to inflammation tissue. With this study, the authors propose serum markers so that the prognosis and diagnosis of non-alcoholic hepatic steatosis become less invasively. Non-alcoholic hepatic steatosis is more frequent in individuals who are diabetic besides obese, and the proteomic analysis of their hepatic metabolic profile reinforces the hypothesis of altered cellular metabolism (β-oxidation, fructose and methionine metabolism) and impairment of mitochondrial function as predisposing factors to this condition. As regards methionine metabolism, the reduction in the levels of the enzymes S-adenosylmethionine synthetase-1 (MAT1), glycine N-methyltransferase (GNMT) and adenosylhomocysteine (SAHH), makes the liver cell more susceptible to damage caused by free radicals, being associated with the development of non-alcoholic steatohepatitis. (Valle *et al*., 2012)

Adipose tissue

Human adipose tissue is divided into two body compartments, the omental adipose tissue and the subcutaneous tissue, which have different metabolic profiles, as analyzed by 2D-DIGE and MALDI-TOF-TOF performed with obese individuals. In relation to subcutaneous adipose tissue, omental adipose tissue presented a proteomic profile more strongly related to inflammatory processes and a compromise with lipolytic activity and glucose metabolism. Thus, the results of these authors corroborate the findings that show the association between excess omental adipose tissue and non-transmissible chronic diseases, such as diabetes and cardiovascular diseases. (Pérez-Pérez *et al*., 2009) When compared to pre-adipocyte secretors of subcutaneous and omental origin, a more pronounced pro-inflammatory component is observed in the omental, represented by the expression of chemoattractant cytokines to monocytes/macrophages, whose tissue infiltration promotes the formation of a pro-inflammatory microenvironment directly associated with the development of metabolic dysfunctions. (Zhu *et al*., 2015) Mature adipocytes derived from subcutaneous adipose tissue demonstrated significant differences when evaluated the metabolic profile of women in different BMI ranges (eutrophic, overweight and grade III obesity). After 2D-DIGE and identification by MALDI-TOF, of the spots with differentiated expression in the overweight groups, 66 were common to both overweight groups, while 23 were of unique expression to the subcutaneous adipose tissue of morbid obese women. When compared to the eutrophic volunteers, differences in metabolic profiles revealed changes in the expression of proteins involved in energy metabolism, in the regulation of antioxidant activity, in inflammation and in cytoskeletal organization. (Benabdelkamel *et al*., 2015) Evaluating subcutaneous adipose tissue, but now comparing young and old obeses, a proteomic analysis revealed a process of resistance to apoptosis in adipocytes, probably due to the chronic exposure of these cells to an environment of chronic inflammation and high oxidizing activity. (Alfadda *et al*., 2013)

Skeletal muscle tissue

Hittel reported an increase in the expression of adenylate kinase 1 (AK1), glyceraldehyde-3-phosphate dehydrogenase (GAPDH) and aldolase A, directly related to overweight, and with possible involvement in the development of insulin resistance. (Hittel *et al*., 2005) Myostatin is a protein of the TGF-β family with a regulatory role in muscle tissue growth and whose expression is increased in extreme obesity and is also related to insulin resistance and skeletal muscle tissue impairment. (Hittel *et al*., 2009) Other markers of skeletal muscle tissue change, such as increased glycolytic proteins and reduced mitochondrial proteins, signal a phenotype of insulin resistance in obese individuals, regardless of whether they are diabetic or not. (Giebelstein *et al*., 2012)

Body biofluid proteom

One of the major limitations in the use of proteomic techniques in the study of diseases is cellular heterogeneity that occurs in pathological processes. This may lead to

Table 1. Relationship between the nutriproteomic approach and the altered processes in the study of obesity

Study	Species	Type of Sample	Proteomic Approach	Processes changed
Rodríguez-Suárez et al. (2010)	Human	Liver	2-DIGE, Maldi-Tof	Chaperone (GRP78) and mitochondrial protein (CPS1)
Valle et al. (2012)	Human	Liver	2-DE, Maldi-Tof	Metabolism of carbohydrates and methionine, mitochondrial enzymes and response to oxidative stress
Alfadda et al. (2013)	Human	Subcutaneous adipose tissue	2-DIGE, Maldi-Tof	Resistance to apoptosis in adipocytes from obese elderly women.
Benabdelkamel et al. (2014)	Human	Subcutaneous adipose tissue	2-DIGE, Maldi-Tof	Carbohydrate and lipid metabolism, tricarboxylic acid cycle, cell signaling, cytostructure regulation, oxidative stress response
Pérez-Pérez et al. (2008)	Human	Subcutaneous and visceral adipose tissue	2-DE e 2-DIGE, Maldi-Tof	Carbohydrate and lipid metabolism, tricarboxylic acid cycle, cell signaling, cytostructure regulation, oxidative stress response
Zhu et al. (2015)	Human	Culture of subcutaneous and visceral adipose tissue cells from biopsies	nanoLC-MS/MS e nanoLC-Orbitrap	Secretion profile most associated with inflammation and tissue remodeling in adipocytes of visceral origin
Giebelstein et al. (2012)	Human	Skeletal muscle	2-DIGE, nano-HPLC/ESI-MS/MS	Glycolysis, mitochondrial and sarcomeric proteins
Hittel et al. (2005)	Human	Skeletal muscle	2-DE, Maldi-Tof	Glycolysis, regulation of energy status
Hittel et al. (2008)	Human	Cultivation of skeletal muscle cells from biopsies	LC-MS/MS	Increased secretion of myostatin by cells from morbidly obese individuals
Martos-Moreno et al (2014)	Human	Plasma	2-DE, MS e Maldi-Tof	Triglycerides, total cholesterol, LDL, HDL, glucose, total insulin, adiponectin
Guzman et al. (2012)	Rats	Blood plasma	Mass spectrometry (UPLC–ESI–qTOF/MS)	Lisofosfatidilcolin, esfingomielin, tetracosahexaenoic acid and 7α-di-hidroxi-4-colestenone.
Leggate et al. (2012)	Human	Plasma e tecido adiposo	One-dimensional poliacrilamida gel electrophorese (1D-PAGE).	Plasma (Interleukin-6, monocyte chemotactic protein and adiponectin) Abdominal tissue (Annexin A2, fatty acid synthetase)
Price et al. (2012)	Mice	Liver	LC-MS/MS	Reduction of hepatic mitochondrial biogenesis
Kalupahana et al. (2010)	Mice	Adipose tissue cell (ATCC 3T3-L1)	2-DIGE, MS	Cycle of tricarboxylic acid, lipogenesis, lipid beta-oxidation
Baiges et al. (2010)	Rats	Liver	nanoLC-MS/MS	Glycolysis, glycogenesis, lipogenesis, via pentose phosphate
Chio et al. (2010)	Rats	Plasma	2-DE, Maldi-Tof	Inflammation, insulin regulation, lipolysis, glucose and vitamin D metabolism, oxidative stress
Joo et al. (2011)	Rats	Adipose tissue	2-DE, Maldi-Tof	Carbohydrate and lipid metabolism, tricarboxylic acid cycle, signal transduction and cell signaling, cytoskeletal regulation

Source: Direct Research, 2015.

inaccurate results, and subsequent examinations are necessary. An example of this is the histopathological evaluation that is performed after the use of tissues. The use of body fluids such as serum, plasma, saliva and urine outweigh these limitations and are more appropriate for longitudinal monitoring. (Ahrens *et al*., 2010) The technical challenges of using fluids in the proteomic model are: the dynamic characteristic of fluid protein composition and the need for analysis of a large number of patients to determine the intra and interindividual variability of a potential marker. (Aps and Martens, 2005) Authors suggest that serum specific protein concentrations may be useful for the early detection of insulin resistance prior to the appearance of other symptoms. This early identification of serum proteins may be potentially important in studies involving obese individuals. (Ding and Mak, 2015; Von *et al*., 2013) In 2014, a cross-sectional study conducted by Martos-Moreno, used the fasting serum sample from a group of obese Caucasian adolescents and compared them with fasting serum samples from the same adolescents after 12 months of follow-up and weight loss. The proteomic analysis used the two-dimensional technique (electrophoresis and mass spectrophotometry). The results showed a decrease in various isoforms of apolipoprotein-A 1, apo J, transthyretin and partially reversed insulin resistance after weight loss. (Martos-Moreno *et al*., 2014) These data suggest that changes in some circulating peptide isoforms accompany the appereance of obesity and may be related to inflammatory processes and insulin resistance, common in obese individuals. Studies of this nature may suggest potential biomarkers that are generally undetectable in routine clinical exams. (Van *et al*., 2010) Mardinoglu, identified several metabolic changes related to obesity in the plasma proteome study of obese individuals. A large number of changes in branched-chain amino acids were observed in obese individuals compared to non-obese individuals. (Mardinoglu *et al*., 2014) The appereance of kidney disease is often associated with obesity. This is usually asymptomatic and insidious, so the use of proteomic techniques in identifying markers has proved to be very useful. Studies have indicated markers of renal injury such as: microalbuminuria, N-acetyl-beta-D-glucosaminidase (NAG), neutrophil-associated, lipocalin gelatinase in obese children. (Ding and Mak, 2015)

Effects of caloric restriction and physical exercise in obesity

Exercise proteomics is an innovative approach that investigates how physical activity has a beneficial role in the fight against obesity. Checking the effects of physical exercises can reveal which proteins can modulate this physical activity, collaborating in a more intense understanding of these physiological mechanisms. It is worth notice that the intensity, frequency and type of exercise should be investigated in detail, since they may alter the expression of these proteins to the detriment of the type of physical activity performed. (Kim *et al*., 2015) A study by Leggate for determination of proteomic changes after two weeks of high intensity training with twelve men with BMI greater than 25 kg/m^2 showed a reduction in the

proteins expression (annexin A2) and adipose tissue synthetase, as a response to physical exercise, as well as reduction of inflammatory cytokines (IL-6, TNF-α) in plasma and adipose tissue. (Leggate *et al*., 2012) Caloric restriction may contribute to the reduction of chronic non-communicable diseases related to body weight, changes in adipose tissue, among others. (Khambatt, 2012) In the study of proteomics, De Guzman JM showed that animals (female mice) maintained in caloric restriction had lower metabolic changes, with a great anti-aging potential. (De Guzman *et al*., 2013) These metabolic changes may be related to the reduction of body fat and oxidative stress. (Genaro *et al*., 2009) According to Price JC, the caloric restriction promotes longevity and it is possible to observe the synthesis and rates of protein degradation through the dynamics of proteomics. (Price *et al*., 2012) This approach allows the identification of proteomic elements related to the state of health and disease.

Dietary treatments and obesity propensity

Although caloric restriction is the main dietary intervention to treat obesity and metabolic syndrome, the propensity for obesity or other dietary interventions (with action in reducing inflammation of adipose tissue regardless of weight loss, for example) are being studied with more details. In the field of nutriproteomics, two studies with cell cultures and with animals treated with functional compounds are highlighted, in addition to 2 studies where the proteomic profile of rats considered resistant or predisposed to obesity were identified.
The proteomic study in cell cultures of adipocytes treated with eicosapentaenoic acid (EPA) showed that numerous proteins involved in the tricarboxylic acid cycle and beta oxidation of fatty acids were at higher levels when compared to cultures treated with arachidonic acid. In addition, lower levels of glycerol-3-phosphate dehydrogenase protein, an important enzyme in lipogenesis, were also observed; which indicates a change in the expression of EPA-induced proteins in vitro necessitating further studies to verify whether it is in vivo.[26]
Modulation was also observed in the protein profile of rats fed with a lipid-rich diet and supplemented with flavonoid extract. In this study of the animals liver proteome, it was observed that more than 140 proteins had a difference of abundance between the animals with high fat diet with and without treatment, and the sugar and lipid metabolisms were particularly altered; confirming that glycogenesis and lipogenesis were again induced by diet and that there is a reversibility caused by the treatment with the functional extract.[27]

On the other hand, the propensity and factors that make humans or other animals susceptible to diet-induced obesity (DIO) are important aspects to consider. Although such factors remain unknown, it is known that humans and rats only develop DIO when they are exposed to a moderately high fat and energy diet; allowing the use of the animal model in studies of the factors that lead to the predisposition and perpetuation of obesity. (Nilsson *et al*., 2012) The proteomic analysis of plasma from obese and thin rats fed with a high fat diet by Choi and Wang revealed that the change in the levels of twelve proteins of varied physiological functions is related to the development of DIO and is mainly observed an increase in the abundance of ITIH4 and a reduction of the Ft B and GSH-Px percussors in rats prone to obesity when compared to the animals considered normal or resistant. (Choi *et al*., 2010) In the adipose tissue analysis by proteomics techniques, 20 proteins were identified that showed significant differences in their regulation among rats that are prone to obesity resistant. Such findings have indicated that brown adipose tissue is more efficiently activated in resistant rats, and that these have a greater use of lipids and a better lipid oxidation capacity. (Joo *et al*., 2011) However, additional studies are needed to directly test the molecular mechanisms and potential functions of each protein identified in obesity and its dietary treatments.

Conclusion

The prospective analysis of representative and well characterized populations to obtain statistical power is one of the limitations of proteomic studies. In addition, few biomarkers have passed the identification phase. Therefore, to increase the relevance of research in Nutriproteomics and in particular, future studies on obesity, will certainly have to focus even more on application to humans. This will be important not only to understand the mode of action of the diet by correlation or association, but to gain a deeper insight into the mechanisms of action through the establishment of causality.

Conflict of interest

Conflict of interest declared none

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