

# Obesity: considerations about etiology, metabolism, and the use of experimental models

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**Abstract:** Studies have been conducted in order to identify the main factors that contribute to the development of obesity. The role of genetics has also been extensively studied. However, the substantial augmentation of obesity prevalence in the last 20 years cannot be justified only by genetic alterations that, theoretically, would have occurred in such a short time. Thus, the difference in obesity prevalence in various population groups is also related to environmental factors, especially diet and the reduction of physical activity. These aspects, interacting or not with genetic factors, could explain the excess of body fat in large proportions worldwide. This article will focus on positive energy balance, high-fat diet, alteration in appetite control hormones, insulin resistance, amino acids metabolism, and the limitation of the experimental models to address this complex issue.

**Keywords:** obesity, diet, leptin, fat, ghrelin, experimental models

## Introduction

Evidence suggests that overweight and obesity prevalence have been arising at alarming rates, both in developing and developed countries. Nearly two-thirds of the adult American population, for example, is already overweight or obese.<sup>1</sup>

Obesity is considered pandemic, as described by the World Health Organization.<sup>2,3</sup> In Brazil, the demographic, economical, and epidemiological changes that took place (intense urbanization process, increased penetration of “western culture,” and globalization, resulting in unfavorable diet and physical activity habits, especially among the poor) in recent years have led to a transition in nutritional patterns: reduction of malnutrition and augmentation of obesity cases.<sup>4-7</sup>

The first, second, and third National Health and Nutrition Examination Surveys (NHANES),<sup>8</sup> conducted in the United States during the years 1971–1974, 1976–1980, and 1988–1991, respectively, showed that in spite of the USD33 million spent by the “weight loss” industry, the number of obesity cases has been rising significantly, with no differences in race and social status. In NHANES II, 25.4% of the adults were considered to be beyond ideal weight (body mass index  $>27.5$  kg/m<sup>2</sup>); in NHANES III this percentage reached 33.3%;<sup>8</sup> in NHANES 1999–2000, this percentage was more than 64%, which was maintained during another study conducted between 2001–2002.<sup>9</sup>

The consequences of obesity to one’s health are many and range from a higher risk of premature death to severe nonlethal disease (comorbidities) associated to it. Obesity is frequently related to hyperlipidemia<sup>10-12</sup> and to type 2 diabetes,<sup>12</sup> two conditions intimately related to cardiovascular diseases.<sup>13-15</sup>

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Ever since complications of obesity to health have been demonstrated, many studies have been conducted in order to identify the main factors that contribute to its development. The role of genetics in the etiology of obesity has also been extensively analyzed. The identification and sequence of the *ob* gene, which encodes the peptide leptin, and the finding that a simple impairment in this gene seems to be the main cause of obesity in *ob/ob* rats<sup>16</sup> have raised the interest in obesity genetics. However, the substantial augmentation of obesity prevalence in the last 20 years cannot be justified by genetic alterations that, theoretically, would have occurred in such a short time.<sup>17,18</sup> Thus, some authors emphasize that the difference in obesity prevalence in various population groups is related to environmental factors,<sup>18,19</sup> especially diet<sup>2,19–21</sup> and the reduction of physical activity.<sup>2,3,19,20,22–24</sup> The aim of this review is to correlate these aspects both in humans and in experimental models, whether or not interacting with genetic factors, in order to try to explain the excess of body fat in large proportions worldwide.<sup>25,26</sup>

## Obesity-inducing factors

### Energy balance

Positive energy balance is an important etiology factor to obesity development, by promoting augmentation in energy storage and body weight.<sup>27,28</sup> Results obtained by the authors' group demonstrated a high sedentary rate in obese subjects. In this study, 80% of the participants did not do any physical activity.<sup>29</sup> In addition, modernization and the economic transition featured in most countries have enhanced processed food intake, which leads to high protein and hyperlipidic diets, and a reduction of complex carbohydrate content.<sup>30</sup> This dietary pattern also seems to be repeating in countries such as Brazil. Studies conducted by the authors' group with obese Brazilian women showed that more than 30% of total caloric intake of this group had been provided by lipids,<sup>31–35</sup> which characterizes a dietary pattern similar to that featured in developed western countries.

In line with this, sedentary life and nutritional habits seem to represent the main risk factors for the etiology of pandemic obesity.<sup>2,6,27</sup> It seems to be the same in Brazil: in 1997, obesity prevalence was estimated to be 11%,<sup>36</sup> while it was around 9.6% in 1989, and 5.7% in 1974.<sup>5</sup> In 1975, there were almost two cases of malnutrition to one of obesity; in 1997 this situation was the exact opposite. Different from other countries, with increasing obesity rates in all social levels, low income for the Brazilian population makes it more susceptible to obesity.<sup>37</sup>

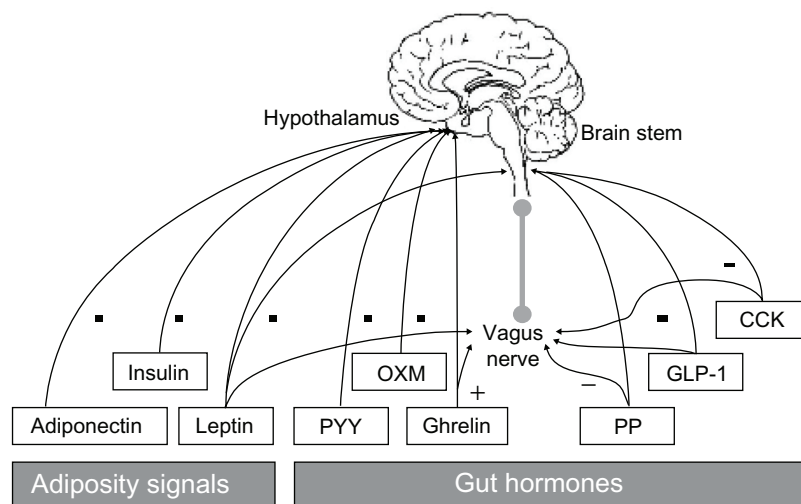
Monteiro et al<sup>38</sup> evaluated the evolution of obesity in Brazil by using data of three national surveys: National Survey of Household Expenditure (ENDEF) 1974, National Health and Nutrition Survey (PNSN) 1989, and the Consumer Expenditure Survey (POF) 2002–2003. In the first 14-year period (1975–1989), obesity rates between men and women increased 92% and 63%, respectively, and the highest rates were found among the low income population. In the second 14-year period (1989–2003), there was an obesity increase even higher in men and a stabilization of this condition in women in general; however among the low income women, there was a 26% increase in obesity. Thus, Monteiro et al highlighted that the distribution of obesity prevalence among the different social classes is especially important.

### Obesity, hyperlipidic diet and appetite-regulating hormones

The literature indicates that not only energy intake and energy expenditure regulate energy body storage. The balance of each nutrient seems to be rigorously controlled to adjust its intake to its oxidation (and vice versa), as recently reviewed by Pereira-Lancha et al.<sup>39</sup>

High-fat diets may lead to hyperphagia or cause metabolic effects regardless of this condition,<sup>40</sup> such as lipolytic activity reduction in fat tissue,<sup>41</sup> reduction in leptin secretion,<sup>42</sup> and/or limitation of leptin capacity.<sup>43</sup> Some studies indicate that impairment on mitochondrial metabolism may lead to excessive body weight gain when the subjects are fed with high-calorie western diets.<sup>44</sup> The study of Townsend et al<sup>45</sup> offered two different diets (hyperlipidic and hypolipidic) to rats, both diets predominant in saturated fat. The animals which consumed a high-fat diet, despite featuring lower total energy intake, had increased body fat more than the low-fat diet group, and had a higher weight gain even with increased leptin levels, pointing to the mechanism of leptin resistance. Townsend et al highlighted that the augmentation of caloric ingestion is not fundamental to stimulating adiposity increase by hyperlipidic diet in rats. Obese individuals feature elevated plasma leptin levels, indicating leptin resistance, which may be caused by impairment in the leptin binding protein that takes leptin to the brain, impairment in the expression and/or signaling of the brain leptin receptor, and impairment in the leptin synthesis/secretion by adipocytes.<sup>46</sup>

Other than leptin, signals provided by the gastrointestinal tract also have an important role in appetite regulation and energy intake (Figure 1); among them are cholecystokinin (CCK), glucagon-like peptide 1, pancreatic polypeptide,



**Figure 1** Evidence suggests that the mechanisms inhibiting both appetite and caloric ingestion may be impaired in obese individuals.<sup>48</sup>

**Note:** This is not yet consensual in the literature, although many authors have been trying to clarify how the mechanisms regulating hunger and satiety behave in this population. Reproduced with the permission of The American Physiological Society from Little TJ, Horowitz M, Feinle-Bisset C. Modulation by high-fat diets of gastrointestinal function and hormones associated with the regulation of energy intake: implications for the pathophysiology of obesity. *Am J Clin Nutr.* 2007;86(3):531–541.

**Abbreviations:** CCK, cholecystokinin; GLP-1, glucagon-like peptide 1; OXM, oxyntomodulin; PP, pancreatic polypeptide; PYY, peptide YY.

all of which have a negative effect on energy balance, and ghrelin, which has the opposite effect.<sup>47</sup>

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## CCK

CCK is one of the most studied hormones regarding food intake regulation. It is largely found in the central nervous system and produced by the jejunum and duodenum as food enters the intestine, especially protein and fat-rich foods, which can increase CCK levels up to five times. When administered, CCK can induce satiety and reduce food portion sizes both in humans and other species in a dose-dependent manner.

The mechanisms by which CCK exerts these effects are controversial. One of the hypotheses is that CCK inhibits gastric peristalsis, mainly by gastric emptying. CCK may also act on vagal afferent nerve fibers to induce cardiovascular effects and reflex inhibition of splanchnic sympathetic nerve discharge.<sup>49</sup> It has been well described that adult rats chronically fed a high-fat diet maintain reduced sensitivity to CCK. Swartz et al<sup>50</sup> demonstrated that male rat pups fed a high-fat diet exhibit reduced sensitivity to CCK; the development of this reduced sensitivity is quicker than its extinction when the high-fat diet was switched to a low-fat diet.

## Peptide YY (PYY)

PYY is secreted by intestine cells after food intake, exerting a negative feedback (inhibits food consumption); however, it has been demonstrated that in obese individuals, PYY levels are lower than in normal people, making this mechanism of satiety less efficient in this case.<sup>51</sup>

Plasma levels of PYY are reduced during a fasted state; they increase gradually approximately 15 minutes after meal initiation. PYY release peak levels occur 1–2 hours after the meal begins and are elevated until 6 hours later. PYY levels are dependent on meal macronutrients and calories, and a protein-rich meal seems to increase PYY levels more than fat-rich or carbohydrate-rich isocaloric meals. In addition, the more protein that is in a diet, the higher the PYY levels.<sup>52</sup> Besides nutrients and calories, bile, hydrochloric acid, vasoactive intestinal polypeptide, and CCK also enhance PYY release.<sup>53</sup> Studies with PYY administration in humans and animals featured both appetite and food intake reduction up to 30% for up to 24 hours. This therapeutic effect might be especially useful in the treatment of obesity, since obese individuals present reduced PYY levels.<sup>54</sup> More and more evidence has demonstrated that PYY has considerable effect on energy expenditure and substrate utilization. Some studies have shown that PYY chronic administration alters substrate utilization favoring fat oxidation.<sup>55</sup> Moreover, overexpressing PYY animals feature higher basal temperature, indicating increased thermogenesis.<sup>56</sup> A study performed with humans comparing eutrophic and obese individuals showed that PYY peripheral infusion increased energy expenditure and fat oxidation.<sup>57</sup>

These data might indicate that the use of this peptide in obesity treatment can be very promising.

### Ghrelin

On the other hand, ghrelin is synthesized and secreted both in the stomach and the intestine, stimulating food intake. Its normal levels are usually low after meals; however, in obese subjects, ghrelin levels are elevated after food intake, stimulating hunger.<sup>58</sup> In fact, some studies evidenced that obese subjects submitted to weight loss treatment demonstrate increased ghrelin levels.<sup>59</sup>

Ghrelin reaches its maximal plasma levels before meals in order to stimulate food intake. After the meal, its levels are reduced to its lowest point approximately 60–90 minutes later, returning to basal parameters. A new release peak occurs a few moments before a new meal.<sup>60</sup> Kinetics of ghrelin plasma concentrations suggest that it is the signal for hunger initiation. Chronic ghrelin administration in rodents results in hyperphagia and body weight gain regardless of ghrelin-induced growth hormone level increases.

Exercise also regulates ghrelin secretion. A study showing the effects of time and intensity of exercise on this parameter showed that subjects who cycled for 120 minutes/day consumed significantly more food compared to those who trained 30 or 60 minutes/day and to the control group.<sup>61</sup>

One of the reasons why diet fat may lead to hyperphagia also comes from its organoleptical properties,<sup>62</sup> such as high palatability, characteristic texture,<sup>21,63</sup> and large utility and versatility as a culinary ingredient.<sup>64</sup> A high-fat diet is preferably consumed even by rats when they can choose among three different chemically defined diets, each of them rich in one of the three primary macronutrients.<sup>65</sup>

However, a few authors believe that the type of diet fat can also influence body fat storage, since there are studies showing significant correlation between body fat percentage and saturated and monounsaturated diet fat percentage.<sup>66</sup> Rats submitted to a high-fat diet for 7 weeks (58% total caloric intake), rich in saturated fat, developed greater adiposity when compared to animals fed with diets rich in omega 3 and omega 6 fats and control animals fed with an isocaloric low-fat diet.<sup>67</sup> Matsuo and Suzuki<sup>68</sup> demonstrated altered affinities of  $\beta$ -adrenergic receptors in brown adipose tissue, the heart, and soleus muscle as a consequence of a high-fat diet rich in saturated fatty acids. Awad and Zepp<sup>69</sup> demonstrated that rats fed with a saturated fatty acid-rich diet featured lower lipolysis rates than animals fed with a polyunsaturated fatty acid-rich diet, due to a lower activity of hormone sensitive lipase. Others have also indicated that saturated fatty acid

intake enhances body fat storage accumulation by a reduction of sympathetic activity in brown adipose tissue, the heart, and skeletal muscle.<sup>68,70</sup>

The activity of carnitine-palmitoyltransferase complex (CPT-1) and, consequently,  $\beta$ -oxidation in brown adipose tissue have also been reported to reduce with the intake of saturated fatty acids.<sup>71</sup> In fact, mitochondrial membrane fluidity, which is determined by a cholesterol/phospholipids rate and by the instauration of the fatty acids that constitute membrane phospholipids, seems to be directly related to CPT-1 activity in the liver.<sup>72</sup>

### Obesity and insulin resistance

Obesity is responsible for triggering a group of metabolic disorders with vascular implications named metabolic syndrome,<sup>73</sup> which is characterized by hyperinsulinemia, high levels of glycemia or diabetes, and at least two different levels of insulin resistance that explain the relationship between various abnormalities and this disease.<sup>74</sup>

Insulin resistance is defined by reduction of its capacity to stimulate glucose utilization,<sup>75</sup> either by insulin deficiency or by impairment in its secretion and/or utilization.<sup>76</sup> There is also an intermediate situation between glucose homeostasis and diabetes named glucose intolerance or reduced glucose tolerance.<sup>76</sup>

Lipid accumulation in the liver is considered to be one of the primary mechanisms relating obesity-related insulin resistance and type 2 diabetes. Glucose uptake is reduced by insulin resistance in muscle and fat cells, whereas insulin resistance in liver cells results in both reduced glycogen synthesis and storage and a failure to suppress glucose production and release into the blood.<sup>77</sup> Abdominal adiposity correlates with hepatic glucose production, and it is also known that free fatty acid flux is increased in obese subjects with upper body adiposity. When free fatty acids are elevated, insulin's ability to suppress hepatic glucose production is markedly attenuated, and this effect is most evident in subjects with impaired glucose tolerance and type 2 diabetes.<sup>77</sup>

Although obesity is pointed at as the most common cause of insulin resistance, not all obese individuals develop this alteration. According to various authors,<sup>78–80</sup> what determines the occurrence of metabolic dysfunction is the location of the accumulation of body fat – mainly visceral adiposity,<sup>80</sup> gender,<sup>81</sup> and low physical activity levels.<sup>82</sup>

It is well established that exercise improves insulin sensitivity. Laaksonen et al<sup>83</sup> studied 612 men who were followed for 4 years. Of these, 107 subjects developed metabolic syndrome, 40% of them exercised less than

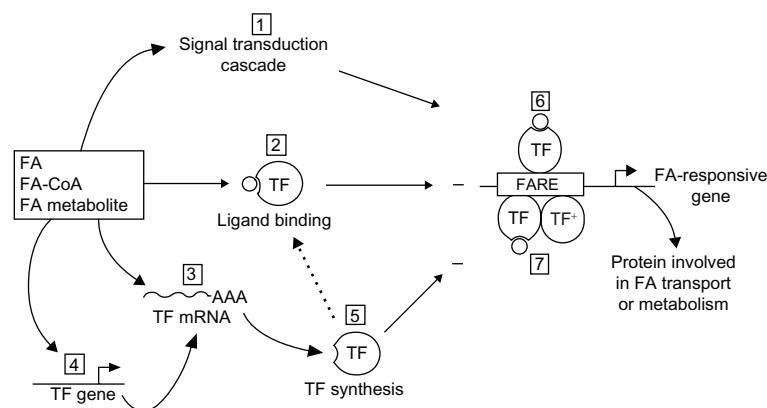
3 hours/week. Other than that, these subjects featured higher blood pressure levels, were heavier, and more dyslipidemic and hyperinsulinemic than the subjects who did not have metabolic syndrome. Laaksonen et al concluded that exercise above the American College of Sports Medicine recommendations strongly reduced the risk of developing metabolic syndrome.

Many studies indicate an augmentation in lipid oxidation when caloric expenditure is higher in obese patients with high-fat intake and elevated rate of lipolysis, which is directly related to body fat storage.<sup>84</sup> This preferential free fatty acid utilization from triacylglycerol storage as an energy substrate would be responsible for reducing glucose mobilization via glycogen. This would lead to a negative feedback of muscle and liver glycogen upon glycogen synthase activity and, consequently, on glycogen storage. Recently, Lancha Jr et al<sup>85</sup> demonstrated the inverse relationship between glycogen content and glucose uptake, ie, the fed animals featured higher glycogen storage and lower glucose uptake whereas the fasted animals had the opposite situation. However, currently, the mechanisms related to glycogen and glucose transport are uncertain; although, some authors believe that glycogen could regulate glucose transport by agglutination of glucose transporter type 4 (GLUT4) molecules and further incorporation into glycogen complex and enzymes. This results in insulin resistance and glucose intolerance; diabetes appears in obese patients after a long period of glucose intolerance and when glycemia does not return to the basal state, there is insulin resistance,<sup>86</sup> which leads to hyperinsulinemia.<sup>87–89</sup>

In rats fed with a high-fat diet, the development of obesity has been observed,<sup>90–93</sup> as previously stated, as well as a reduction in systemic, muscle, and adipocyte insulin action.<sup>90–95</sup> Raubenheimer et al<sup>96</sup> fed rats with a 45% fat diet for 8 weeks and observed an increase in body weight, triacylglycerol levels of 117%, hyperinsulinemia, and glucose intolerance. Nascimento et al<sup>97</sup> demonstrated that animals fed with hyperlipidic diets featured lower phosphorylation of one of the proteins of the insulin signaling mechanisms (protein kinase B/Akt), a denotation of reduced insulin action, and resistance induced by diet.

High-fat diet intake has been given much attention, especially in regards to its effects on insulin action alteration. These alterations have already been observed in a very short period of time, for example, 7 days,<sup>92</sup> 10 days,<sup>91</sup> 3 weeks,<sup>98</sup> and 4 weeks.<sup>95</sup> Various studies demonstrated that insulin resistance occurs primarily in the liver, in a very short time (3 days), followed by insulin action impairment in some groups of skeletal muscles.<sup>99,100</sup> These effects depend on the type of diet fat (amount, size, and number of unsaturations).<sup>101</sup> The prolonged exposition of adipocytes to saturated fatty acids has caused insulin resistance in these cells.<sup>102</sup> Many mechanisms have been proposed to explain the action of lipids in gene expression control of both GLUT4 and insulin receptors. Tebbey et al<sup>103</sup> suggested that arachidonic acid (n = 6) reduces the stability of GLUT4 messenger ribonucleic acid, lowering their levels rapidly.

According to Duplus et al,<sup>104</sup> fatty acid molecules may act in various moments of gene transcription. Figure 2 shows some possible mechanisms to illustrate this. For example,



**Figure 2** Postulated mechanisms for fatty acid control of gene transcription.<sup>104</sup> The FA per se, FA-CoA, or FA metabolite modulate (±) transcription of a responsive gene, encoding a protein involved in FA transport or metabolism, through various non-mutually selective potential mechanisms. Step 1: a signal transduction cascade is initiated to induce a covalent modification of a TF, thereby modifying its transcriptional potency. Step 2: the FA itself or its derivative acts as a ligand for a TF, which then can bind DNA at a FA response element and activate or repress transcription. Steps 3, 4 and 5: FA can act indirectly via alteration in either TF mRNA stability (Step 3) or gene transcription (Step 4), resulting in variations of de novo TF synthesis (Step 5) with an impact on the transcription rate of genes encoding proteins involved in FA transport or metabolism. On binding to the eognate response element, TF acts either as a monomer (Step 6), a homodimer, or a heterodimer with TF<sup>+</sup>, a different TF (Step 7).

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**Abbreviations:** FA, fatty acids; FA-CoA, fatty acyl-CoA; TF, transcription factor.



fatty acids could initialize a signaling cascade, in a covalent way, to modulate the transcription factor. Another possibility would be the liaison of fatty acid to the transcription factor to activate or inhibit this process. Finally, fatty acids could modify messenger ribonucleic acid stability, as stated before, and also alter de novo synthesis of the transcription factor, altering protein synthesis.

Another hypothesis to explain gene expression modulation by hyperlipidic diets is related to nuclear receptors like peroxisome proliferator-activated receptors (PPARs), which act like transcription factors. It has been demonstrated that PPARs aid glucose uptake regulation via adiponectin, a protein expressed mainly by white adipose tissue. Their levels are reduced in various types of obesity and insulin resistance.<sup>79</sup> High-fat diet intake also seems to exert effects upon gene expression of PPAR  $\gamma$  coactivator 1 $\alpha$ . PPAR  $\gamma$  coactivator 1 $\alpha$  consists of a PPAR coactivator, and both have a strict relationship with adipocyte synthesis and apoptosis and with increased insulin sensitivity. Sparks et al's study<sup>105</sup> demonstrated that after 3 days of diet, animals featured a reduction in PPAR  $\gamma$  coactivator 1 $\alpha$  gene expression of approximately 25%.

Mammalian target cells of rapamycin are possibly enrolled in the mechanisms of insulin resistance, as indicated by various in vitro studies, through its signaling pathway.<sup>106</sup> Nevertheless, whether this interference is significant or not in vivo is still a controversial point. Reynolds et al<sup>107</sup> recently demonstrated that a hyperlipidic diet rich in saturated fat caused insulin resistance in rats. However, this situation was not reversed in the presence of rapamycin, a powerful and specific mammalian target of rapamycin inhibitor. Thus, Reynolds et al concluded that the progression of insulin resistance in rats, resulting from a high-fat intake, was not dependent on mammalian target of rapamycin activation in skeletal muscle.

Adipose tissue is an important source of proinflammatory cytokines and adipokines. It is known that subclinical inflammation in obese individuals may be a consequence of elevated cytokine secretion by adipocytes, contributing to the development of metabolic syndrome.<sup>80</sup> Adipokines and cytokines secreted by white adipose tissue include leptin (appetite inhibitor and T cell proliferation modulator); adiponectin (potent vascular protector associated to adequate glucose uptake, with concentration inversely proportional to adiposity);<sup>79</sup> resistin (gives resistance to insulin); and tumor necrosis factor  $\alpha$  (TNF- $\alpha$ ; reduces adiponectin production).<sup>108</sup> According to some authors, TNF- $\alpha$ , a transmembrane protein, could also explain alterations in gene expression of molecules

evolved in glucose uptake, mediated or not by insulin, after a high-fat diet intake. TNF- $\alpha$  may act on the insulin signaling cascade, phosphorylizing insulin receptor substrate 1 serine residues, which impairs its binding capacity to the insulin receptor and inhibits signal propagation.<sup>109</sup>

In addition to its effect on the insulin signaling cascade, some studies indicated that TNF- $\alpha$  is capable of reducing GLUT4 gene expression.<sup>110</sup> Jove et al<sup>111</sup> studied the effect in vitro of saturated fat on TNF- $\alpha$  gene expression and verified that there was an augmentation of 2.5 times in both gene and protein expression, which was inversely correlated to GLUT4 levels and glucose uptake. Borst et al<sup>112</sup> administered an antiTNF- $\alpha$  substance to Sprague Dawley rats and verified an increase of more than 60% in muscle glucose transport. The high-fat diet utilized in this study has already been associated to a TNF- $\alpha$  level increase in Wistar rats.<sup>113</sup> Thus, it is possible that TNF- $\alpha$  might have contributed, at least in part, to the modification of glucose tolerance in this study. However many of these hypotheses are not demonstrated yet and more studies are necessary to completely understand the alterations in gene expression of various proteins after high-fat diets.

Many studies have tried to identify the causes of glucose uptake alterations due to high-fat diets; nevertheless this issue is still controversial. One of the hypotheses is that alteration of glucose receptors impair insulin action, mainly by reduction of GLUT4 activity. For example, Zierath et al<sup>114</sup> found impairment in GLUT4 activity in muscle tissue after high-fat diet consumption in rats.

## Interrelations between obesity and amino acid metabolism

Some authors believe that amino acids could have important implications in insulin postreceptor mechanisms, impairing GLUT4 vesicle translocations.<sup>115</sup> Lancha Jr<sup>116,117</sup> found reduced glucose transport in skeletal muscles of Wistar rats supplemented with aspartate and asparagine (both 45 mg/kg body weight/day) for 5 weeks when compared to the control group. Tyrosine kinase is an enzyme responsible for structural modifications of insulin receptor substrate 1 during the events of the first cell cycle. No alteration in tyrosine kinase activity has been found in supplemented rats;<sup>116,118</sup> amino acids may have interfered in any of the numerous postreceptor episodes.

An elevated fat intake and low carbohydrate consumption found in obese patients may be responsible for elevating free fatty acid plasma levels and, consequently, increasing body fat storage in adipose tissue.<sup>119</sup> Hyperlipidic diets promote

an increase of CPT-1 activity in liver and muscle tissue, augmenting lipid oxidation.<sup>120</sup> According to Liu et al,<sup>121</sup> one of the factors that increases muscle CPT-1 activity, due to high-fat diets rich in unsaturated fatty acids, is the reduction of CPT-1 sensitivity to malonyl-coenzyme A (CoA). Synthesized from acetyl-CoA carboxylation, malonyl-CoA is critical in the regulation of lipid metabolism, providing acetyl-activated groups to fatty acid chain synthesis and inhibiting CPT-1 and, consequently,  $\beta$ -oxidation. The high intake of unsaturated fatty acids would augment mitochondrial membrane fluidity, reducing CPT-1 inhibition by malonyl-CoA.<sup>121</sup> The fatty acid flux increases towards mitochondrion and, according to Koves et al,<sup>122</sup> is not accompanied by complete  $\beta$ -oxidation due to the incapacity of the tricarboxylic acid (TCA) cycle to adjust to increased oxidative demand. Thus, metabolites would accumulate in its interior, leading to mitochondrion stress and insulin resistance. Nevertheless, a study conducted by Bruce et al<sup>123</sup> demonstrated that when an overexpression of CPT-1 in muscle tissue is produced, impaired insulin sensitivity due to a high-fat diet improved. Bruce et al had already found an increase in CPT-1 activity, insulin resistance, triacylglycerols, and fat storage in cell membranes associated with a high-fat diet. According to them, obesity as a consequence of a fat-rich diet results in an increase of lipids entering the cell with a subsequent increase in oxidation. However, an increase in fat oxidation is quantitatively lower than lipids entering the cell, and results in higher fat deposits in the cell. The accumulation of these bioactive lipid molecules as ceramides and diacylglycerols might modify lipid characteristics of the cell membrane, interfering in insulin action. However, when promoting CPT-1 overexpression in muscle tissue, insulin resistance was reduced. Thus, Bruce et al suggested that fat entering the mitochondrion is more critical in regulating fatty acid oxidation than  $\beta$ -oxidation and the TCA cycle.

In line with this, for this process to be triggered and the TCA cycle to run normally, there has to be an oxaloacetate yield in the same proportion as acetyl-CoA. In regular conditions, glycogen is more relevant initially, and plasma glucose has a more discrete participation; afterwards, when a reduction in the storage occurs, plasma glucose assumes this function of oxaloacetate provision by glycolysis and further conversion of pyruvate to oxaloacetate.<sup>124</sup> Nevertheless, when glucose/glycogen concentration is reduced due to fasting or low carbohydrate intake, or when resulting from insulin resistance for example, oxaloacetate yield becomes deficient. Amino acids are processed in muscle in order to correct this deficiency; leucine, isoleucine, valine, aspartate,

and asparagine may be metabolized in muscle tissue to generate TCA cycle intermediates (such as succinate and oxaloacetate) to maintain its flux. These amino acids donate their carbon chains to form intermediates and release ammonia in muscle tissue. Table 1 indicates the amino acids metabolized directly by muscles, the enzymes responsible for transferring amino groups, and the intermediates formed.

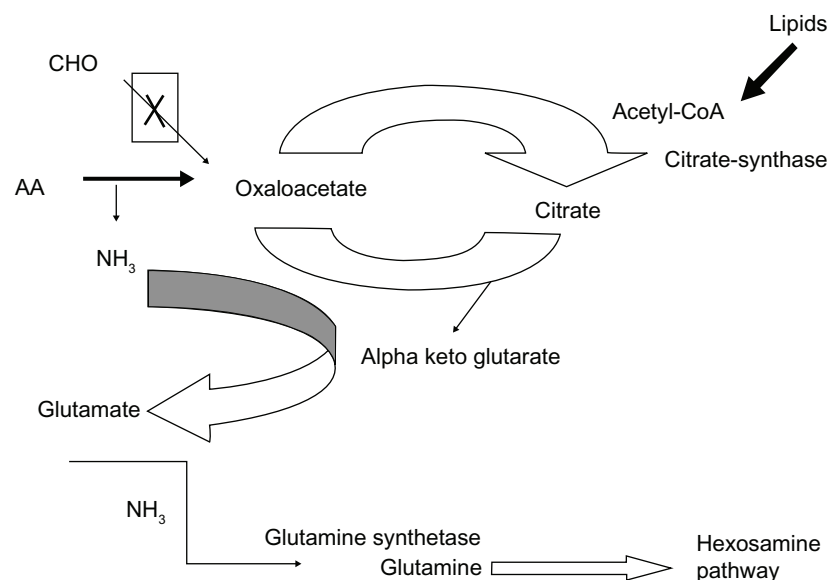
As long as the TCA cycle is maintained, there will be an increase in citrate levels that will inhibit phosphofructokinase,<sup>124</sup> reducing glucose utilization and, as a consequence, glucose uptake. This is one of the hypotheses suggested by some authors to explain the reduction in glucose uptake and, consequently, insulin resistance. Another possibility suggested by Marshall et al,<sup>125</sup> among others, to clarify insulin resistance in this situation is the glycosylation of postinsulin substance receptors due to an increase in glucosamine levels, which is a product of the hexosamine pathway. In this case, ammonia that is liberated from muscle cells by amino acid metabolism would be shifted to  $\alpha$ -ketoglutarate, which is a TCA cycle intermediate, generating glutamate. Glutamate and ammonia would be metabolized by glutamine synthase, resulting in glutamine synthesis. Glutamine is usually utilized as an energy source by the intestine and immune cells. Nevertheless, a high glutamine production, as a consequence of the elevated TCA cycle activity, would stimulate the hexosamine pathway (Figure 3). In this pathway, glutamine and fructose 6-phosphate generate glucosamine 6-phosphate and other products in a reaction catalyzed by glutamine: fructose 6-phosphate amino transferase.<sup>126</sup>

The augmentation of amino acid metabolism, which generates oxaloacetate and other intermediates to the Krebs cycle, implies elevation of the cell oxidative capacity and may also provoke other cell structural alterations (Figure 4).<sup>127</sup> Other studies also evidenced structural changes related to amino acid supplementation, and the authors justified these results as a consequence of an increase cell protein turnover<sup>128</sup>

**Table 1** Amino acid processes in muscle metabolism and their respective enzymes, yielding tricarboxylic acid cycle intermediates

Amino acids	Enzymes	Intermediates
Aspartate	Aspartate aminotransferase	Oxaloacetate
Asparagine	Asparagine aminotransferase	Oxaloacetate
Isoleucine	Leucine aminotransferase	Acetyl-CoA and Propionil-CoA
Leucine	Leucine aminotransferase	Acetyl-CoA
Valine	Valine aminotransferase	Succinate

**Abbreviation:** CoA, coenzyme A.



**Figure 3** Adaptation of muscle metabolism to a high availability of lipids.

**Note:** Reduced participation of carbohydrates and high amino acid participation in anaplerotic reactions are observed, activating the hexosamine pathway.

**Abbreviations:** AA, amino acid; CHO, carbohydrate; CoA, coenzyme A; NH<sub>3</sub>, ammonia.

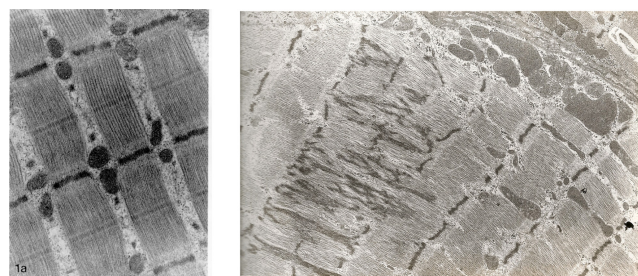
or of mitochondrion calcium flux augmentation due to the increase in mitochondrion size and number, which could stimulate calcium-dependent proteases.<sup>129</sup> However, few studies have discussed the relationship between body fat accumulation and amino acid metabolism in order to verify the possible modifications in amino acid metabolic pathways caused by an elevated fat intake.

## The central nervous system “feeding” hypothesis

One of the hypotheses that can be explained by the reduction in glucose utilization as a result of insulin resistance, as described above,<sup>124</sup> is to ensure glucose availability to central nervous system cells. In normal individuals, long-term signals of lipid cell metabolism, such as leptin

and insulin levels, are regulated by body fat stores; these hormones modulate central effectors of energy balance by regulating central nervous system responses to short-term meal-related signals.<sup>130</sup> In obese individuals, these mechanisms are blunted by either genetic or environmental reasons, for example, increased efferent parasympathetic activity in the endocrine pancreas, mediated by the vagus nerve, was observed early in genetically obese rats (fa/fa) who received arginine infusion, resulting in higher insulin secretion compared to lean animals.<sup>131</sup> On the other hand, Nogueiras et al<sup>132</sup> found that obese mice lost the capacity of modulating adipocyte metabolism through glucagon-like peptide 1 system, which is one of the functions controlling fat deposition, enhancing insulin release, and reducing both glucagon levels and gastric emptying via neuroendocrine mechanisms.

In general, obese individuals’ diets are characterized by excessive caloric ingestion, mainly due to larger protein and lipid intake and, proportionally, lower carbohydrate ingestion. For this reason, carbohydrate stores may not be available to the tissues in the same intensity as the other two macronutrients, and the increase in insulin levels may be interpreted as an attempt to modulate peripheral hormonal responses to augment glucose uptake for the central nervous system. For instance, daily fluctuations in energy balance (towards a deficit) in premenopausal women seem to be the most influent factor in changing ovarian estrogen exposure and sex hormone-binding



**Figure 4** Micrograph of mitochondrial impairment caused by aspartate and asparagine supplementation in a rat model.

**Notes:** The left panel shows the soleus muscle of the sedentary control group (15,000×) and the right panel shows the soleus muscle of the supplemented group (aspartate and asparagine) (7000×).<sup>117</sup>



globulin levels, which correlate with a reduction in insulin level.<sup>133</sup> Maybe the opposite takes place in obese subjects: increased insulin levels reduce sex hormone-binding globulin levels,<sup>134</sup> which are correlated to increased abdominal fat and the presence of metabolic alterations in men and women.<sup>135</sup>

## Experimental models of obesity

The study of obesity in humans would probably answer many questions; however, these kinds of studies have several ethical and financial limitations. Experimental studies allow a greater quantity of inquiries and results, and feature more controlled diet and health conditions where the animals can be kept free of pathogens. Although experimental obesity models cannot be compared to human obesity models, they are of great value in the study of biochemical, physiological, and pathological conditions necessary for the accumulation of excessive body fat. In the last 20 years, knowledge about various factors leading to obesity and its metabolic and endocrine consequences has been increasing. Much of this was generated by experimental obesity models.<sup>136</sup>

Many forms of genetically inherited obesity are described in the literature.<sup>137</sup> Rodents such as Sprague Dawley rats are particularly propitious to obesity caused by high-fat (cafeteria) diets, due to their polygenic heritage. Some authors believe that this is an appropriate and realistic model to study human obesity.<sup>138,139</sup> However, some alterations found in human obesity do not occur in these genetic animal models, for example, an increase in fat-free mass and some hormonal differences.<sup>140</sup> Thus, it is clear that the study of obesity in genetically altered animals has several limitations; some models can only be used for evaluation of specific obesity alterations, but are inappropriate for other investigations.

Other than that, due to the fact that a rapid increase in obesity cases worldwide is related to the lack of physical activity<sup>141,142</sup> and higher food availability and intake,<sup>2,142,143</sup> many studies have been performed using the so-called “nongene obesity models” which consist of increasing food intake in laboratory animals.<sup>144</sup> However, in rodents, for example, it is hard to elevate caloric intake spontaneously, even when the diet is flavored.

Obesity development is possible though, even without a caloric intake increment, because a change in nutrient composition or in dietary patterns influences the efficiency of food utilization and, consequently, increases fat storage per consumed calorie. In practical terms, an increase in diet density may result in caloric intake augmentation or a raise in caloric ingestion of a determined macronutrient, resulting

in an increase of body fat. High-carbohydrate diets and/or high-fat diets, as well as a cafeteria diet, have also been used to develop obesity in rats.

A cafeteria diet consists of fat-rich foods often found in coffee shops and supermarkets (eg, cookies, salami, cheese, bread) and many experimental studies in rats have used this diet protocol. This technique is aimed at mimicking the food patterns of modern diets, which consist of many cafeteria and fast food meals in experimental conditions, characterizing a high-fat diet named “western diet.”<sup>2,5,19,21,34</sup> In fact, some studies demonstrate an increase in fat intake in animals fed with this kind of food;<sup>137</sup> however, these studies have limitations. In a pilot study performed in the authors’ laboratory, evaluating and controlling the animal intake of different kinds of food was difficult. In the experimental group, food intake was extremely heterogeneous and hard to evaluate, since 1 day after making the food available to the animals in individual cages, it was not possible to separate each meal to calculate both their intake in terms of macronutrients and the type of fat consumed. This aspect has been the object of much controversy, mainly in studies of diet-induced thermogenesis.<sup>140,145–147</sup>

An increase in body fat storage can be attained by augmenting diet fat intake; however it is advisable to avoid a reduction in protein/energy rate in order to avoid the impairment of growth and development of the experimental animals. In practical terms, diets containing 60% of fat and 30% of protein initially used butter and eggs; lard and casein have been used more recently in order to reduce the cost of food preparation. This composition also allows the high intake of saturated fatty acids similar to that observed in western diets. The most recent Consumer Expenditure Survey published by the Brazilian Institute of Statistics and Geography found that lipids contributed 27.6%, 31.5% of which consisted of saturated fat, to household food consumption.<sup>148</sup> Results found by the authors’ group indicated a high intake of saturated fatty acids in the diet of obese women: 62.4% of lipid intake was related to saturated fatty acids (unpublished observations), which corresponds to 17% of total caloric value. According to the American Heart Association,<sup>149</sup> fatty acid ingestion should not exceed 7% of daily total caloric intake. In an attempt to develop experimental models that enable studies of obesity and its consequences, this high saturated fat intake is interesting, since the contribution of this type of fat in the impairment of insulin sensitivity due to obesity has been demonstrated.<sup>102</sup> This alteration in plasma insulin levels seems to be highly related to other associated

pathologies, such as hypertension, dyslipidemia,<sup>150</sup> and atherosclerosis.<sup>11</sup>

## Conclusion

More studies are necessary to elucidate the environmental factors and molecular mechanisms that induce obesity, especially in Brazil, since the low-income population seems to be more vulnerable to this disease and will lead to enormous health cost increases in the future.

The link between amino acid metabolism and insulin resistance, among others, leads to a fertile field for new information on obesity etiology. Experimental models are important in obesity research due to more controlled experimental conditions, although care must be taken to extrapolate this data to human subjects.

It is crucial to stimulate physical activity and dietary pattern change through both education and government strategies worldwide.

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The authors report no conflicts of interest in this work.

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