Effect of Hyperbaric Oxygen Therapy (HBOT) on Insulin Resistance Associated with Abdominal Obesity in Wistar Rats with Dietary Sucrose-Induced Metabolic Syndrome

Sinuhé Raúl Cruz-Villanueva1, Julio César Ramírez-Nava1, Juan Antonio Moreno-Luna1, Karime Guadalupe Cárdenas-Ureña1, Luz Teresa Espín-Iturbe4, María Guadalupe Sánchez Otero2, Rodolfo Quintana-Castro1,2 and Alfonso Alexander-Aguilera1,2*

1 Escuela de Posgrados de Sanidad Naval, Hospital Naval de Especialidades de Veracruz, Secretaría de Marina, Gral. Figueroa No. 151, Col. Faros Centro, Veracruz, Ver., 91700, México
2 Facultad de Bioanálisis, Universidad Veracruzana, Carmen Serrán s/n. Col. Flores Magón, Veracruz, Ver., 91700, México
3 Escuela de Medicina, Universidad Cristóbal Colón, Carr. Veracruz-Medellín s/n. Col. Puente Moreno, Boca del Río, Ver., 94271, México
4 Facultad de Medicina, Universidad Veracruzana, Carmen Serrán s/n. Col. Flores Magón, Veracruz, Ver., 91700, México

(Received December 24, 2020)

Summary Metabolic syndrome (MS) is a combination of risk factors that contribute to the development of mainly type 2 diabetes mellitus, cardiovascular disease (CVD) and nonalcoholic fatty liver disease (NAFLD). Its prevalence has increased worldwide, and healthcare systems will face major challenges in addressing this problem. The aim of this work was to evaluate the effect of hyperbaric oxygen therapy (HBOT) on insulin resistance (IR) and obesity associated with MS in Wistar rats. The experimental design consisted of three groups of sucrose-induced MS rats: the MS group that consumed sucrose (MS-Suc; n = 5), the MS group that ingested sucrose and HBOT (MS-Suc-HBOT; n = 5), the MS group that did not consume sucrose and that received HBOT (MS-HBOT; n = 5) and the control group. The rats received HBOT for 20 d at 2.4 atmospheres absolute (ATA) for 60 min. Subsequently, the rats were euthanized, and body fat weight, serum biochemical parameters and microscopic analysis of adipose tissue were determined. Rats with hyperoxia had decreased body weight, adipose tissue hypertrophy, and abdominal and epididymal fat. Likewise, markers of insulin resistance (glucose, insulin and HOMA-IR), biochemical parameters of dyslipidemia (cholesterol and triglycerides) and nonalcoholic fatty liver (AST and ALT) decreased; in contrast, compared to the control group, HBOT increased the 1/HOMA-IR, HOMA-βCell and McAuley indexes, which were related to the improvement in insulin sensitivity (p<0.05; p<0.01). HBOT showed beneficial effects in the treatment of IR and obesity associated with sucrose-induced metabolic syndrome in Wistar rats.

Key Words hyperbaric oxygen, metabolic syndrome, obesity, insulin resistance, diabetes

Metabolic syndrome (MS) is a combination of risk factors that contribute to the development of type 2 diabetes mellitus (DM2), cardiovascular diseases (CVDs) and nonalcoholic fatty liver disease (NAFLD), among other diseases; this syndrome is a pathological condition that includes insulin resistance associated with abdominal obesity, hypertension and hyperlipidemia (1).

The prevalence of MS has been steadily increasing worldwide (2): in the Western population, it ranges from 28.5% to 38.5% (3–5), while in the Eastern population, the prevalence is only 24.1%. Based on the above, the world’s health systems will face major challenges to address this public health problem (6).

One of the most important pathophysiological events in the progression of obesity to MS and DM2 is insulin resistance (IR), which is defined as an impaired response of the body to insulin; IR alters glucose metabolism in the body, causing a compensatory increase in insulin production or hyperinsulinemia. The metabolic consequences of IR associated with visceral obesity include hyperglycemia, hypertension, dyslipidemia, hyperuricemia, elevated inflammatory markers, endothelial dysfunction and prothrombotic state (7–9).

Due to the importance of MS as a health problem, great efforts have been made to prevent obesity associated with IR; however, in the presence of damage, different healing strategies have been addressed. In recent years, the use of hyperoxia or hyperbaric chamber oxygen therapy (HBOT) has emerged, in which 100% oxygen is applied with increased atmospheric pressure, usually 2.4 atmospheres absolute (ATA) (10, 11). This therapy has been used for the treatment of pathologies
related to oxygen depletion at the cellular and tissue levels, such as carbon monoxide poisoning, gas embolism, soft tissue infections, and in the healing of lower limb wounds in diabetic patients at risk of amputation (12, 13).

The main mechanism of HBOT is thought to be related to the cellular response to oxidative stress; research reports have shown that hyperoxia decreases the production of inflammatory mediators and oxygen free radicals (14). These protective effects can be attributed to the inhibition of reactive oxygen species (ROS) formation, which alters a wide variety of cellular mechanisms, such as cell signaling pathways (15–17).

The development of abdominal obesity in MS is characterized by adipocyte hypertrophy and inflammation due to the accumulation of triglycerides. This association is related to insulin resistance in different tissues and the presence of dyslipidemia. Adipose tissue inflammation is accompanied by the alteration of different cellular processes, such as modifications in oxidative stress that have a direct relationship with metabolism and the cellular processes involved in the regulation of gene expression. For this reason, providing a hyperoxic cellular environment could reverse the pathophysiological events of MS, such as insulin resistance, obesity and dyslipidemia. Based on the above, the aim of the present work was to evaluate the effects of HBOT on insulin resistance and obesity associated with MS in a Wistar rat model induced with sucrose ingestion.

**MATERIALS AND METHODS**

*Induction of the MS model.* Thirty nearly 21-d-old Wistar rats were purchased from Envigo RMS, Inc. The rats were housed individually using stainless steel cages and maintained on a 12-h light/dark cycle at an ambient temperature of 25˚C. Animal maintenance and handling were performed in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

The rats were divided into two groups: the metabolic syndrome-sucrose group (MS-Suc, n = 20), which received a standard diet (Envigo RMS, Inc.) plus 30% sucrose in the drinking water as an MS inducer for 20 wk, and the control group (group C; n = 10). After experimental treatment with sucrose, five rats from each group were euthanized, and their body weight, adiposity index and MS parameters were determined.

**Evaluation of the effects of hyperbaric oxygen therapy on MS.** Once the animal model of MetS was obtained, the effects of HBOT were evaluated. The animals were divided into four groups, which received a standard diet (Envigo RMS, Inc.) or without sucrose plus HBOT: 1) a metabolic syndrome group, which received sucrose as a disease inducer (MS-Suc; n = 5); 2) a group with metabolic syndrome previously developed with sucrose intake and to which sugar was stopped, plus treatment with hyperbaric oxygen (MS-Suc-HBOT; n = 5); 3) a metabolic syndrome group without sucrose consumption plus hyperbaric oxygen treatment (MS-HBOT; n = 5) and 4) a control group (group C, n = 5).

The rats that received HBOT were treated with 2.4 atmospheres absolute (ATA) for 60 min for a period of 20 continuous days. For this purpose, a small animal hyperbaric chamber, IYOCA brand, model CAME02, was used. HBOT was applied at the same time in the morning to exclude the possible effect on the circadian rhythm. The Naval Hospital of Veracruz, Mexico, has fully operational hyperbaric facilities for studies in humans and animal models.

After treatment with hyperoxia for 20 consecutive days, the rats were sacrificed after sedation. Variables such as blood weight, food intake, body fat (abdominal and epididymal), and adiposity index were obtained, and serum parameters (glucose, insulin, uric acid, triglycerides, cholesterol, AST and ALT) were obtained. Some organs were removed (liver, brain and heart), and adipose tissue was preserved in neutral buffered formalin until microscopic analysis.

**Blood samples.** During sacrifice, blood samples were obtained from animals fasted for 18 h. The blood was centrifuged at 1,086 × g for 10 min, and the serum was stored at −20˚C until analysis.

**Serum biochemical parameters.** Serum levels of glucose, cholesterol, triglycerides, aspartate aminotransferase (AST), alanine aminotransferase (ALT), uric acid and other serum markers were measured by enzymatic methods and automated Selectra-E equipment. Serum free fatty acids (FFAs) were determined from frozen samples by an enzymatic method (NEFA-C assay, FUJIFILM Wako Pure Chemical Corporation). The serum insulin concentration was analyzed by enzyme-linked immunosorbent assay (ELISA kits).

**Indicators of insulin resistance or sensitivity.** The following formula was used to calculate the HOMA-IR index: insulin (µU/mL) × glucose (mM/L) / 22.5 (18); and for the reciprocal of insulin: 1/HOMA-IR (19, 20). For the HOMAβ-cell index, the following formula was used: 20 [fasting serum insulin (µU/mL)/[fasting plasma glucose (mM/L) − 3.50]] (21, 22); and for the McAuley index: exp [2.63 × 0.28 ln (fasting insulin in µU/mL) − 0.31 ln (fasting triglycerides in mM/L)] (23).

**Microscopic analysis.** Adipose tissue samples were fixed for 24 h in 10% neutral buffered formalin. Subsequently, the tissue was embedded in paraffin. Tissue sections (4–6 µm thick) were stained with hematoxylin-eosin for microscopic examination. Direct observation of at least 15 fields at low magnification was performed; digital images were obtained using an Olympus BX51 microscope equipped with a Camedia C3040ZOOM digital camera (Olympus America Inc., Corporate Center Drive, Melville, NY, USA). All images were recorded at 10×, 20× and 40× magnification.

**Data analysis.** The normality and homogeneity of variance of the experimental results were checked. ANOVA and Tukey’s test, which is a multiple range test for the comparison of means (p < 0.05 or p < 0.01), were performed; the results are expressed as the means and SD of five replicates. The results were analyzed with the
RESULTS

Dietary caloric intake, body weight and adipose tissue gain

Table 1 presents the dietary caloric intake of rats in the control group and those that consumed sucrose or the experimental group (MS-Suc group) during 1, 10 and 20 wk of metabolic syndrome induction. Rats in the MS-Suc group showed a caloric increase when drinking sucrose-containing water, whereas solid diet intake decreased due to the caloric contribution of sugar ($p < 0.05$). However, both groups received the same final caloric intake (isocaloric diet) during the experimental treatment period.

Figure 1 shows the evolution of the body weight of the rats that consumed sucrose for 20 wk to induce metabolic syndrome, while Table 2 shows that the rats in the MS-Suc group showed a final body weight gain of 38% and a body gain of 71% compared to the control rats. The rats of both groups presented the same feeding efficiency; however, it is important to mention that the source of caloric intake of the diets was different and that the MS-Suc group received sucrose as an inducer of metabolic syndrome ($p < 0.05$) (Table 2).

In relation to total body fat, the rats that consumed sucrose showed increases of 83% in body fat (abdominal and epididymal fat) and 32% in the adiposity index compared to the control group ($p < 0.05$). This phenomenon was related to alterations in serum parameters; triglycerides increased by 95% and uric acid by 20%, while HDL decreased by 48%, and there was no difference in glucose levels compared to the control group ($p < 0.05$) (Table 2).

Minitab 17 statistical package.

Table 1. Liquid consumption and food and caloric intake in sucrose-fed (MS-Suc group) and control (C group) rats during 20 wk of experimental treatment.

| Parameter            | 1 wk          | 10 wk         | 20 wk         |
|----------------------|---------------|---------------|---------------|
|                      | C Group       | MS-Suc Group  | C Group       | MS-Suc Group  | C Group       | MS-Suc Group  |
| Liquid consumption   |               |               |               |               |               |               |
| mL/d                | 47.40±3.60    | 31.60±2.40    | 123.10±36.90  | 54.70±16.39   | 149.90±22.30  | 49.96±7.43    |
| mL/d/100 g bw       | 61.96±2.40*   | 61.96±2.40*   | 83.59±12.39*  | 83.59±12.39*  | 73.58±10.97*  |               |
| kcal in drinking water |               |               |               |               |               |               |
| g/d                 | 65.00±3.86    | 49.72±11.16*  | 46.20±11.74*  | 45.00±14.80   | 19.87±6.08*   |               |
| g/d/100 g bw        | 43.33±2.57    | 29.24±6.56*   | 15.40±3.91*   | 15.00±4.93    | 4.78±1.46*    |               |
| kcal in food        | 176.35±10.45  | 119.00±26.29* | 146.26±28.30  | 168.49±13.63  | 61.05±20.06   |               |
| Total kcal/d/100 g bw | 176.35±10.45 | 180.96±28.69  | 146.26±28.30  | 168.49±13.63  | 146.26±28.30  |               |

Values are mean±SD (C group, n=5; MS-Suc group, n=15). * $p<0.01$.

Total kcal/d/100 g bw= kcal in drinking water + kcal in food.
bw = body weight.
Evaluation of HBOT in metabolic syndrome

Once the Wistar rat model with sucrose-induced metabolic syndrome was established, the effects of hyperbaric oxygen therapy (HBOT) were analyzed.

The final body weight of the rats that received sucrose without HBOT (MS-Suc group) showed an increase of 24% with respect to the rats in the control group, while the rats that received sucrose and HBOT presented an increase of 14% but showed a decrease of 20% with respect to the MS-Suc groups; however, the rats that received HBOT without sucrose showed a decrease in body weight of 20% compared to the rats without HBOT. Therefore, the latter group of rats obtained similar values to the control group (p<0.05).

In relation to total body fat, it was found that the rats in the MS-Suc group without HBOT showed an increase of 62% in comparison to the control group, while those located in the MS-Suc-HBOT and MS-HBOT groups showed decreases in body fat of 35% and 43%, respectively, in comparison to the control group (p<0.05), showing values equal to control group. In the same sense, the MS-Suc group obtained an increase in abdominal fat of 25% with respect to the control group, while the MS-Suc-HBOT and MS-HBOT groups reached values similar to the control group; thus, the rats of the MS-Suc-HBOT and MS-HBOT groups showed decreases of 35% and 43% of abdominal fat, respectively (p<0.05), with respect to the MS-Suc group. Similarly, rats that consumed sucrose with HBOT showed a 476% increase in epididymal fat compared to control rats; however, rats from the MS-Suc-HBOT and MS-HBOT groups showed 71% and 82% decreases in this type of fat, respectively (p<0.05), with respect to the MS-Suc group.

In relation to the above, the body fat index increased in the rats that ingested sucrose without HBOT by 30% compared to the control rats; however, the rats that received HBOT in the MS-Suc-HBOT and MS-HBOT groups had 29% decreases in the body fat index in both groups, obtaining values similar to the control group. Finally, there were no changes in the weights of some organs, such as the liver and heart, or in the hepatosomatic and cardiosomal indexes, which presented the same percentages in all groups in the experimental design (Table 3).

Table 2. Body weight, feed efficiency, adiposity index and serum biochemical parameters in control (C) and sucrose (MS)-fed rats at 20 wk of experimental treatment.

|                     | Control Group | MS-Suc Group |
|---------------------|---------------|--------------|
| Initial body weight (g) | 148±20        | 155±22       |
| Final body weight (g)   | 300±35        | 415±30**     |
| Body gain (g)           | 152±17        | 260±18**     |
| Feed efficiency (%)     | 4.44±0.66     | 3.41±0.51    |
| Total body fat (g)      | 20.40±1.17    | 37.53±1.76**|
| Adiposity index         | 6.80±0.72     | 9.04±0.42*   |
| Glucose (mg/dL)         | 151.00±11.72  | 158.00±6.43  |
| Triglycerides (mg/dL)   | 105.00±3.00   | 205.00±10.00*|
| HDL (mg/dL)             | 60.61±6.66    | 31.74±4.00*  |
| Uric acid (mg/dL)       | 1.02±0.01     | 1.23±0.02*   |

Values are the mean±SD (C group, n=5; MS-Suc group, n=15).

*p<0.05, **p<0.01.
Total body fat = Abdominal fat (g) + Epididymal fat (g).
Adiposity index = (Total body fat/final body weight) 100.
Feed efficiency = (Body gain/food consumed) 100.

Table 3. Body parameters and organ weights of rats in the control, MS-Suc, MS-Suc-HBOT and MS-HBOT groups during 20 d of experimental treatment in the hyperbaric chamber.

|                     | Control Group | MS-Suc Group | MS-Suc-HBOT Group | MS-HBOT Group |
|---------------------|---------------|--------------|-------------------|---------------|
| Initial body weight (g) | 300±35        | 415±30*      | 420±25*           | 410±15*       |
| Final body weight (g)   | 436±10*       | 542±21*      | 498±22*           | 435±19*       |
| Weight gain (g)         | 136±15*       | 127±9*       | 78±5*            | 25±2*         |
| Abdominal fat weight (g) | 20.18±1.08*   | 25.27±1.11*  | 20.16±1.48*      | 18.33±1.36*   |
| Epididymal fat weight (g) | 1.82±0.77*    | 10.50±0.67*  | 3.00±1.85*       | 1.96±0.47*    |
| Total body fat (g)      | 22.00±1.85*   | 35.77±1.78*  | 23.16±8.33*      | 20.29±6.95*   |
| Adiposity index          | 5.04±0.42*    | 6.59±0.32*   | 4.65±0.46*       | 4.66±0.69*    |
| Liver weight (g)        | 11.62±1.70*   | 14.46±0.72*  | 12.64±0.88*      | 12.13±2.84*   |
| Hepatosomatic index      | 2.66±0.30*    | 2.67±0.31*   | 2.53±0.17*       | 2.78±0.65*    |
| Heart weight (g)        | 1.12±0.14*    | 1.39±0.69*   | 1.08±0.13*       | 0.97±0.09*    |
| Cardiosomatic index      | 0.25±0.05*    | 0.26±0.04*   | 0.22±0.04*       | 0.22±0.05*    |

Values are the mean±SD (C group, n=5; MS-Suc group, n=5; MS-Suc-HBOT group, n=5; MS-HBOT group, n=5).

*p<0.05, *p<0.01, **p<0.001.
Total body fat = Abdominal fat (g) + Epididymal fat (g).
Adiposity index = (Total body fat/final body weight) 100.
Hepatosomatic index = (Liver weight/final body weight) 100; Cardiosomatic index = (Heart weight/final body weight) 100.
and 31%, and urea decreased by 36% and 23% \((p < 0.05)\). Finally, the AST/ALT index increased in the two groups of rats receiving HBOT with respect to the MS-Suc group \((p < 0.05)\).

Regarding insulin resistance, rats consuming sucrose without HBOT had 40% higher insulin levels and an 85% higher HOMA-IR index than control rats, while these parameters decreased by 43% and 44% in the MS-Suc-HBOT group and by 55% and 85% in the MS-HBOT group, respectively; thus, rats that received hyperoxia obtained similar values with respect to the control group \((p < 0.05)\) (Fig. 2).

In relation to insulin sensitivity, rats receiving HBOT with and without sucrose consumption (MS-HBOT-Suc and MS-HBOT groups) showed increases in the reciprocal insulin resistance \((1/\text{HOMA-IR})\) of 43% and 86% \((p < 0.05)\) and increases in the McAuley index of 22% and 44%, respectively, whereas there was no change in

Table 4. Serum biochemical parameters in rats of the control group and the metabolic syndrome group (MS-Suc group) and rats that received hyperbaric chamber treatment (MS-HBOT-Suc and MS-HBOT groups) for 20 d.

| Parameters          | Control Group | MS-Suc Group | MS-Suc-HBOT Group | MS-HBOT Group |
|---------------------|---------------|--------------|-------------------|--------------|
| Urea (mg/dL)        | 44.80±5.97a   | 61.21±3.06b  | 39.00±10.77a      | 47.00±4.08a  |
| Creatinine (mg/dL)  | 0.78±0.11a    | 1.01±0.05b   | 0.79±0.50a        | 0.70±0.09a   |
| Uric acid (mg/dL)   | 1.88±0.03a    | 2.56±0.02b   | 2.44±0.05c        | 2.27±0.05d   |
| Cholesterol (mg/dL) | 63.60±17.60a  | 86.89±4.34b  | 56.40±6.43a       | 59.50±10.08a |
| Triglycerides (mg/dL)| 160.60±24.09a| 219.42±10.97b| 181.20±10.00a    | 120.50±18.07d|
| AST (U/L)           | 50.01±6.00a   | 68.33±7.00b  | 55.16±6.50a       | 48.84±5.00a  |
| ALT (U/L)           | 37.15±4.00a   | 245.99±11.00b| 150.78±5.00a      | 54.99±5.00a  |
| AST/ALT             | 1.34±0.15a    | 0.27±0.01b   | 0.36±0.11c        | 0.88±0.08d   |

Values are the mean±SD (C, \(n=5\); MS-Suc group, \(n=5\); MS-Suc-HBOT group, \(n=5\); MS-HBOT group, \(n=5\)).

\(a,b,c\) \(p<0.05\).
the HOMA-β cell index. All the above results were compared with respect to the SM-Suc group, whereby rats receiving hyperoxia showed equal values compared to control rats (p<0.05) (Fig. 3).

**Microscopic analysis of adipose tissue**

Figure 4 shows the histopathological analysis of abdominal adipose tissue. The control group presented mature tissue without evidence of inflammation or hypertrophic adipocytes (Fig. 4A). In contrast, the MS-Suc group consuming sucrose and without HBOT presented hypertrophic adipocytes (Fig. 4B). Conversely, the groups that received HBOT exhibited important tissue changes, and the MS-Suc-HBOT group showed mature tissue without inflammation but presented some large adipocytes (Fig. 4C); however, the MS-HBOT group without sucrose intake presented a normal histological pattern (Fig. 4D) that was similar to that of the control group.

**DISCUSSION**

Rats that consumed sucrose in drinking water as an inducer of MS (MS-Suc group) developed obesity, which was characterized by an increase in body weight of 38% with respect to the control group, and showed an increase in body fat (abdominal and epididymal fat) of 83%. These results are consistent with those reported by other authors who have used sucrose or fructose as dietary inducers of metabolic disorders in rats, such as insulin resistance, obesity and MS (24–29). Experimental evidence has shown that the consumption of sucrose increases caloric intake and body weight. The mechanism involved is related to the consumption of sucrose or fructose in liquid form, both in the short (30) and long term (31). This physiological process is associated with reduced satiety due to the inability to stimulate the production of leptin and other hormones that regulate appetite, tending to increase caloric intake during subsequent meals (32–34) and favoring the development of obesity.

In relation to the above, rats that consumed sucrose (MS-Suc group) developed large amounts of abdominal and epididymal fat. Regarding this adipose tissue storage phenomenon, some authors have reported that visceral and intramuscular fat accumulation is a primary factor in the pathogenesis of various metabolic disorders, including insulin resistance, type 2 diabetes, and hyperlipidemia (35–38). However, abdominal obesity is the most important risk factor for the development of MS and nonalcoholic fatty liver disease (NAFLD) (39, 40). In humans, adipose tissue produces and secretes a large number of proinflammatory adipocytokines that cause adipose tissue inflammation, which is associated with the development of metabolic disorders such as type 2 diabetes mellitus and NAFLD.

Regarding the effects of HBOT or hyperoxia on MS, it was found that rats subjected to hyperbaric oxygen and consuming sucrose as an inducer of SM (MS-HBOT-Suc group), as well as those in which sucrose consumption was suspended (MS-HBOT group), presented a decrease in body fat. Rats in the MS-Suc-HBOT group decreased abdominal fat by 35% and epididymal fat by 71%, while in the MS-HBOT group, abdominal fat decreased by 43% and epididymal fat by 82%. These changes were accompanied by a significant decrease in insulin resistance. Rats in the MS-Suc-BOTH group showed a 43% decrease in hyperinsulinemia and rats in the MS-HBOT group showed a 55% decrease according to the results of the HOMA-IR criterion; therefore, these rats showed an increase in sensitivity to this hormone, taking into account the results of the reciprocal of 1/HOMA-IR resistance. On the other hand, some markers of dyslipidemia (triglycerides and cholesterol) increased, and there was an increase in the AST/ALT index, which is related to NAFLD. The effects of HBOT were significant in rats consuming sucrose as an inducer of MS; however, it is important to note that HBOT applied to rats without sucrose ingestion had better beneficial effects in the treatment of MS.

The effects of hyperoxia described above have been studied by different research groups, establishing the relationships between the effect of oxygen at the cellular level and the different possible molecular mechanisms. The most important cellular processes related to HBOT are 1) oxidative stress; 2) oxidation of molecules that could activate cell signaling cascades; 3) increased secretion of enzymes or hormones; 4) regulation of gene expression; 5) changes in organelle functionality; and 6) activation or inhibition of metabolic pathways for energy production or thermogenesis, among others (11). These results revealed the beneficial effects of HBOT; however, it is important to perform more studies to determine the curative or detrimental effects of HBOT since some authors report collateral effects due to increased production of reactive oxygen compounds (ROS) in the liver (41).

In relation to obesity, hyperglycemia and insulin sensitivity in rats that received HBOT (MS-HBOT-Suc and
MS-HBOT groups), it has been reported that hyperbaric oxygen improves skeletal muscle oxidative metabolism and inhibits adipocyte hypertrophy in type 2 diabetic animals with obesity. The latter is due to increases in metabolism and mitochondrial function in muscle fibers that prevent the transition of fibers from slow to fast, inhibiting glucose elevation and adipocyte hypertrophy (42). The normalization of glucose described above could be related to a decrease in damage to the β-cells of the pancreas caused by this sugar, allowing adequate insulin secretion, which together with increased muscle metabolism significantly improves insulin sensitivity.

Regarding the metabolic events between decreased adipose tissue hypertrophy (Fig. 4) and improved insulin sensitivity (Fig. 2), these results are related to lipid metabolism, reducing dyslipidemia in rats that received HBOT. The MS group receiving simultaneous HBOT and succrose (MS-HBOT-Suc group) showed a decrease in the triglyceride levels of 17% and in cholesterol of 35%, whereas rats receiving HBOT without sucrose consumption (MS-HBOT group) showed greater decreases in triglyceride levels of 45% and cholesterol of 31%.

During lipid metabolism, triglycerides in adipocytes are enzymatically hydrolyzed to glycerol and free fatty acids (FFAs), and these molecules are released into the blood (42) and transported to the liver and undergo β-oxidation for oxygen-dependent mitochondrial respiration; this phenomenon decreases excess triglycerides in adipose tissue. The results for the triglyceride and insulin levels are related to the results of the McAuley index, which relates serum triglycerides and insulin to measure the sensitivity to this hormone. In this sense, significant increases in the McAuley index (23) of 22% for rats in the MS-HBOT group and 44% for rats in the MS-HBOT group were found in comparison with rats with metabolic syndrome without HBOT (p<0.05). The above shows the relationship between adipose tissue lipolysis, insulin sensitivity, hypertriglyceridemia and hyperoxia, the latter of which could favor β-oxidation and lipid metabolism.

Some reports have established that regulation of the expression of genes associated with fat oxidation in adipocytes causes MS reversal. PPAR-γ and PGC1-α proteins increase fatty acid oxidation, which is associated with improved insulin sensitivity in adipose tissue due to increased oxygen consumption by the process of lipolysis resulting in decreased ectopic fat accumulation inhibiting the development of obesity (43–45).

MS is associated with the development of NAFLD; it has been reported in humans and animal models that the fatty acids produced by lipolysis of adipose tissue are transported to the liver, favoring the accumulation of lipids causing steatosis (46); one of the indicators used is an AST/ALT ratio less than 1. Hyperoxia decreased the serum levels of hepatic aminotransferases in rats receiving HBOT and increased the AST/ALT ratio; these results show the beneficial effect on liver damage, which could be related to increased mitochondrial oxidation of fatty acids in β-oxidation (42); however, further studies are needed to determine the specific molecular mechanism.

In the present study, a series of events demonstrated the beneficial effects of HBOT or hyperoxia on MS in rats. 1) decreased body weight and abdominal and epidymal fat, 2) decreased adipose tissue hypertrophy. 3) reversal of insulin resistance and increased insulin sensitivity, and 4) normalization of the diagnostic biochemical parameters of hyperinsulinemia, dyslipidemia, and NAFLD.

In conclusion, HBOT or hyperoxia had beneficial effects in the treatment of MS in Wistar rats, mainly significantly decreasing body weight and abdominal fat, which was associated with reversal of insulin resistance (HOMA-IR) and normalization of serum biochemical markers of dyslipidemia and NAFLD.

Disclosure of state of COI

There are no conflicts of interest.

REFERENCES

1) The international Diabetes Federation. The IFD Consensus Worldwide Definition of the Metabolic Syndrome: International Diabetes Federation: Belgium. 2006.
2) Alberti KG, Eckel RH, Grundy SM, Zimmet PZ, Cleeman JJ, Donato KA, Fruchart JC, James WP, Loria CM, Smith SC, Jr. International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute. American Heart Association, World Heart Federation, International Atherosclerosis Society. International Association for the Study of the Obesity Harmonizing the metabolic syndrome: a joint interim statement of the International Diabetes Federation Task Force on Epidemiology and Prevention, National Heart, Lung, and Blood Institute; American Heart Association; World Heart Federation; International Atherosclerosis Society; and International Association for the Study of Obesity. 2009. Circulation 120(16): 1640–1645.
3) Li Y, Zhao L, Yu D, Wang Z, Ding G. 2018. Metabolic syndrome prevalence and its risk factors among adults in China: A nationally representative cross-sectional study. PLoS ONE 13: e0199293.
4) Ford ES, Li C, Zhao G. 2010. Prevalence and correlates of metabolic syndrome based on a harmonious definition among adults in the US. Diabetes Care 33: 180–193.
5) Dunbar JA, Reddy P, Davis-Lameloise N, Philpot B, Laatikainen T, Kilkkinen A, Laatikainen T, Kilkkinen A, Banker S, Vartiainen E, Lo SK, Janus ED. 2008. Depression: An important comorbidity with metabolic syndrome in a general population. Diabetes Care 31: 2368–2373.
6) Li R, Li WC, Lun ZJ, Zhang HP, Sun Z, Kanu JS, Qiu S, Cheng Y, Liu Y. 2016. Prevalence of metabolic syndrome in mainland China: A meta-analysis of published studies. BMC Public Health 16: 296.
7) Seong J, Kang JY, Sun JS, Kim KW. 2019. Hypothalamic

Authorship

AAA and JCRN: Research conception and design. SRCV, KGCU: Administration of hyperbaric oxygen therapy to rats. JAML: Histological analysis. LTEI: Establishment of the sucrose-induced metabolic syndrome model. MGSO: Determination of serum biochemical parameters. RQC: Statistical analysis.
inflammation and obesity: a mechanistic review. Arch Pharm Res 42: 383–392.

8) Brown JC, Harhay MO, Harhay MN. 2019. The value of anthropometric measures in nutrition and metabolism: Comment on anthropometrically predicted visceral adipose tissue and blood-based biomarkers: A cross-sectional analysis. Nutr Metab Insights 12: 1–3.

9) Deacon CF. 2019. Physiology and pharmacology of DPP-4 in glucose homeostasis and the treatment of type 2 diabetes. Front Endocrinol (Lausanne) 10: 80.

10) Gill AL, Bell CN. 2004. Hyperbaric oxygen: its uses, mechanisms of action and outcomes. QJM 97: 385–395.

11) Thom SR. 2011. Hyperbaric oxygen: its mechanisms and efficacy. Plant Reconst Surg 127: 1318–1418.

12) Wattel F. 1998. European Committee Consensus Conference on Hyperbaric Oxygen in the Treatment of Foot Lesions in Diabetic Patients. ECHM. Glaxo-Welcome, France.

13) Feldmeier JJ. 2003. Hyperbaric oxygen: indications and results. The Hyperbaric Oxygen Therapy Committee Report. Undersea and Hyperbaric Medical Society. Maryland, USA.

14) Lin HC, Wan FJ, Wu CC, Wu TH. 2005. Hyperbaric oxygen protects against lipopolysaccharide-stimulated oxidative stress and mortality in rats. Eur J Pharmacol 508: 249–254.

15) Herrlich P, Bohmer FD. 2000. Redox regulation of signal transduction in mammalian cells. Biochem Pharmacol 59: 35–41.

16) Rosette C, Karin M. 1996. Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science 274: 1194–1197.

17) Dalton TP, Shertzer HG, Puga A. 1999. Regulation of gene expression by reactive oxygen. Annu Rev Pharmacol Toxicol 39: 67–101.

18) Matthews DR, Hosker JP, Rudenski AS, Naylor BA, Treacher DF, Turner RC. 1985. Homeostasis model assessment: insulin resistance and beta-cell function from fasting plasma glucose and insulin concentrations in man. Diabetologia 28: 412–419.

19) Yokoyama H, Emoto M, Fujisawa S, Motoyama K, Morioka T, Komatsu M, Tahara H, Shoji T, Okuno Y, Nishizawa Y. 2003. Quantitative insulin sensitivity check index and the reciprocal index of homeostasis model assessment in normal range weight and moderately obese type 2 diabetic patients. Diabetes Care 26: 2426–2432.

20) Yokoyama H, Emoto M, Fujisawa S, Motoyama K, Morioka T, Komatsu M, Tahara H, Shoji T, Inaba M, Nishizawa Y. 2004. Quantitative insulin sensitivity check index and the reciprocal index of homeostasis model assessment are useful indexes of insulin resistance on plasma triglyceride responses. J Clin Endocrinol Metab 94: 2471–2480.

21) Turner RC, Rudenski AS, Matthews DR, Levy JC, O’Rahilly SP, Hosker JP. 1990. Application of structural model of glucose-insulin relations to assess beta-cell function and insulin sensitivity. Horm Metab Res 24: 66–71.

22) Li Y, Xu W, Liao Z, Yao B, Chen X, Huang Z, Hu G, Weng JP. 2004. Induction of long-term glycemic control in newly diagnosed type 2 diabetic patients is associated with improvement of beta-cell function. Diabetes Care 27: 2597–2602.

23) McAuley KA, Williams SM, Mann JI, Walker RJ, Lewis-Barned NJ, Temple LA, Duncan AW. 2001. Diagnosing insulin resistance in the general population. Diabetes Care 24: 460–464.

24) Johnson, RJ, Segal MS, Sautin Y, Nakagawa T, Feig DI, Kang DH, Gersch MS, Benner S, Sanchez-Lozada LG. 2007. Potential role of sugar (fructose) in the epidemic of hypertension, obesity and the metabolic syndrome, diabetes, kidney disease, and cardiovascular disease. Am J Clin Nutr 86: 899–906.

25) Olliart RR, Torres-Marquez ME, Badillo A, Angulo GO. 2001. Dietary fatty acids effects on sucrose-induced cardiovascular syndrome in rats. J Nutr Biochem 4: 207–212.

26) Bunag RD, Tomita T, Sasaki S. 1983. Chronic sucrose ingestion induced mild hypertension and tachycardia in rats. Hypertension 5: 218–225.

27) Wright DW, Hansen RI, Mondon CE, Reaven GM. 1983. Sucrose-induced insulin resistance in the rat: modulation by exercise and diet. Am J Clin Nutr 38: 879–883.

28) Hwang Y, Ho H, Hoffman BB, Reaven GM. 1987. Fructose-induced insulin resistance and hypertension in rats. Hypertension 10: 512–516.

29) Solano SM, Buzán-de Santillana I, Soto RL, Christian Bautista PC, and Alexander AA. 2018. Tissue changes in the development of fatty liver by chronic ingestion of sucrose associated with obesity and dyslipidemia in rats. Int J Vitam Nutr Res 88: 117–125.

30) Toff KL, Grudziak J, Townsend RR, Dunn TN, Grant RW, Adams SH, Keim NL, Cummings BP, Stanhope KL, Havel PJ. 2009. Endocrine and metabolic effects of consuming fructose- and glucose-sweetened beverages with meals in obese men and women: influence of insulin resistance on plasma triglyceride responses. J Clin Endocrinol Metab 94: 1562–1569.

31) Rezvani R, Cianflone K, McGahan JP, Berglund L, Bremer AA, Keim NL, Griffen SC, Havel PJ, Stanhope KL. 2013. Effects of sugar-sweetened beverages on plasma acylation stimulating protein, leptin and adiponectin: relationships with metabolic outcomes. Obesity 21: 2471–2480.

32) DiMeglio DP, Mattes RD. 2000. Liquid versus solid carbohydrate: effects on food intake and body weight. Int J Obes Relat Metab Disord 24: 794–800.

33) Havel PJ. 2005. Dietary fructose: implications for dysregulation of energy homeostasis and lipid/carbohydrate metabolism. Nutr Rev 63: 133–157.

34) Morton GJ, Cummings DE, Baskin DG, Barsh GS, Schwartz MW. 2006. Central nervous system control of food intake and body weight. Nature 443: 289–295.

35) Cornier MA, Dabelea D, Hernandez TL, Lindstrom RC, Steig AJ, Stob NR, Van Pelt RE, Wang H, Eckel RH. 2008. The metabolic syndrome. Endocr Rev 29: 777–822.

36) Ide T, Kobayashi H, Ashakumary L, Rouyer IA, Taka-hashi Y, Aoyama T, Hashimoto T, Mizugaki M. 2000. Comparative effects of perilla and fish oils on the activity and gene expression of fatty acid oxidation enzymes in rat liver. Biochem Biophys Acta 1485: 23–35.

37) Roche HM. 2005. Fatty acids and the metabolic syndrome. Proc Nutr Soc 64: 23–29.

38) Simopoulos AP. 2008. The importance of the omega-6/
omega-3 fatty acid ratio in cardiovascular disease and other chronic diseases. Exp Biol Med 233: 674–688.

39) de Santa M, Olalla L, Sánchez-Muñiz FJ, Vaquero MP. 2009. N-3 fatty acids in glucose metabolism and insulin sensitivity. Nutr Hosp 24: 113–127.

40) Lin DS, Connor WE. 1990. Are the n-3 fatty acids from dietary fish oil deposited in the triglyceride stores of adipose tissue? Am J Clin Nutr 51: 535–539.

41) Tsuneyama K, Chen Y-Ch, Fujimoto M, Sasaki Y, Suzuki W, Shimada T, Iizuka S, Nagata M, Abursta M, Chen S-Y. 2011. Advantages and disadvantages of hyperbaric oxygen treatment in mice with obesity hyperlipidemia and steatohepatitis. ScientificWorldJournal 11: 2124–2135.

42) Fujita N, Nagatomo F, Murakami S, Kondo H, Ishihara A, Fujino H. 2012. Effects of hyperbaric oxygen on metabolic capacity of the skeletal muscle in type 2 diabetic rats with obesity. ScientificWorldJournal 2012: 637978.

43) Ebbert JO, Jensen MD. 2013. Fat depots, free fatty acids, and dyslipidemia. Nutrients 5(2): 498–508.

44) Roberts LD, Ashmore T, Kotwica AO, Murfitt SA, Fernandez BO, Feeish M, Murray AJ, Griffin JL. 2015. Inorganic nitrate promotes the browning of white adipose tissue through the nitrate-nitrite-nitric oxide pathway. Diabetes 64(2): 471–484.

45) Goodpaster BH, Kelley DE, Wing RR, Meier A, Thaete FL. 1999. Effects of weight loss on regional fat distribution and insulin sensitivity in obesity. Diabetes 48(4): 839–847.

46) Sheedfär F, Sung MMY, Aparicio-Vergara M, Kloosterhuis NJ, Miquilena-Colina ME, Vargas-Castrillón J, Febrerio M, Jacobs RL, Alain de Bruin, Vinciguerra M, García-Monzón C, Holker MH, Dyck JRB, Koonen DPY. 2014. Increased hepatic CD36 expression with age is associated with enhanced susceptibility to nonalcoholic fatty liver disease. Aging (Albany NY) 6(4): 281–295.