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ORIGINAL RESEARCH ARTICLE

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Whole body ARHGAP21 reduction improves glucose homeostasis in high-fat diet obese mice

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GTPase activating proteins (GAPs) are ubiquitously expressed, and their role in cellular adhesion and membrane traffic processes have been well described. TBC1D1, which is a Rab-GAP, is necessary for adequate glucose uptake by muscle cells, whereas increased TCGAP, which is a Rho-GAP, decreases GLUT4 translocation, and consequently glucose uptake in adipocytes. Here, we assessed the possible involvement of ARHGAP21, a Rho-GAP protein, in glucose homeostasis. For this purpose, wild type mice and ARHGAP21 transgenic whole-body gene-deficiency mice (heterozygous mice, expressing approximately 50% of ARHGAP21) were fed either chow (Ctl and Het) or high-fat diet (Ctl-HFD and Het-HFD). Het-HFD mice showed a reduction in white fat storage, reflected in a lower body weight gain. These mice also displayed an improvement in insulin sensitivity and glucose tolerance, which likely contributed to reduced insulin secretion and pancreatic beta cell area. The reduction of body weight was also observed in Het mice and this phenomenon was associated with an increase in brown adipose tissue and reduced muscle weight, without alteration in glucose-insulin homeostasis. In conclusion, the whole body ARHGAP21 reduction improved glucose homeostasis and protected against diet-induced obesity specifically in Het-HFD mice. However, the mechanism by which ARHGAP21 leads to these outcomes requires further investigation.

KEYWORDS
ARHGAP21, glucose homeostasis, insulin secretion, obesity, Rho-GAP

1 | INTRODUCTION

GTPase activating proteins (GAPs) increase the intrinsic GTPase activity of small G proteins accelerating their return to an inactive state (Bos, Rehmann, & Wittinghofer, 2007; Lamarche & Hall, 1994). Small G proteins are classified into five principal families: the Ras, Rho, Rab, Arf, and Ran, according to their sub-domains (Wennerberg, Rossman, & Der, 2005) and, in turn, each family has specific GAPs (Bos et al., 2007). Indeed, these proteins play an important role in cytoskeletal rearrangements, membrane trafficking and cell cycle regulation in several cell types (Bishop & Hall, 2000; Hall, 1998; Kowluru, 2010; Van Aelst & D'Souza-Schorey, 1997). All these events suggest a possible role of GAP proteins in glucose homeostasis. However, few of them have been explored in this context.

The most studied GAP on glucose homeostasis is the Rab-GAP TBC1D1. Its inhibition in skeletal muscle cells impairs glucose uptake

(Chadt et al., 2015; Stöckli et al., 2015; Szekeres et al., 2012). When it is inhibited in primary rat beta cells, meanwhile, glucose-stimulated insulin secretion increases (Rütti et al., 2014), reinforcing the importance of these proteins in glucose-insulin homeostasis.

Only recently, the GAPs from the Rho family have been explored in this context, for example, the overexpression of TCGAP decreases glucose uptake in adipocytes (Chiang et al., 2003), and the inhibition of ARHGAP21 in islets from neonatal mice increases insulin secretion (Ferreira et al., 2015). The ARHGAP21 is a Rho-GAP that controls cell proliferation, migration (Bigarella et al., 2012), differentiation (Bassères, Tizzei, Duarte, Costa, & Saad, 2002), cell-cell adhesion (Perillo et al., 2015), and cell-cell junction remodeling (Barcellos et al., 2013; Sousa et al., 2005). Its function has been highlighted in cancer studies, where it acts as a tumor suppressor (Bigarella, Borges, Costa, & Saad, 2009; Luo et al., 2016). However, the possible effect of ARHGAP21 on glucose homeostasis has not been yet determined. Thus, we used ARHGAP21-haplodeficient mice aiming to explore this question.

Here, we observed that whole body ARHGAP21 reduction in Het-HFD mice improved glucose tolerance and insulin sensitivity, which was associated with reduced insulin secretion. Body weight was also reduced in both, the Het and Het-HFD groups. However, it seems that the type of diet consumed affects the mechanism by which ARHGAP21 impacts body weight, as Het-HFD mice displayed reduced white adipose tissue, whereas Het mice showed increased brown adipose tissue and lower muscle weight.

Taken together, we confirmed the involvement of Rho-GAPs in glucose homeostasis, and described an as-yet unknown role for these proteins in controlling white and brown fat depots and muscle mass. These findings suggest the Rho-GAP ARHGAP21 protein as an important modulator of glucose and energetic metabolism, supporting future investigations to explore the mechanism by which this phenomenon occurs.

2 | MATERIALS AND METHODS

2.1 | Ethics statement

All the experiments described herein were approved by the State University of Campinas Committee for Ethics in Animal Experimentation (approval number: 3783–1) and performed according to the "Principles of laboratory animal care" (NIH publication no. 85–23, revised 1985).

2.2 | Animals

The ARHGAP21 transgenic mice (Het) are a whole-body gene-deficiency model (heterozygous, expressing approximately 50% of ARHGAP21). The generation and genotyping of ARHGAP21-haplo-deficient mice were performed as previously described (Xavier-Ferrucio et al., 2017). Paired male wild type littermates served as controls (Ctl). All mice were maintained at standard housing conditions, on a 12-hr light–dark cycle at 22 ± 1 °C. At 1-month-old, the mice were fed ad libitum with a chow diet (Ctl and Het) or a high-fat diet, (Ctl-HFD and Het-HFD) for 10 weeks. Diet compositions were previously described (Batista et al., 2013).

2.3 | Body parameters and blood measurements

The body weight of all mice was evaluated during 10 weeks after the beginning of chow or high-fat diet introduction. At the end of this period, the mice (10 hr fasting) were euthanised in a CO_2-saturated chamber followed by decapitation and the blood samples were collected. Afterward, these blood samples were centrifuged at 1,100 g, 4 °C for 15 min, to obtain the serum, which was stored at −20 °C to posterior insulin measurements by radioimmunoassay (RIA) (Scott, Atwater, & Rojas, 1981). In addition, the perigonadal and subcutaneous fat pads, interscapular brown adipose tissue, and the gastrocnemius skeletal muscle were removed and weighed.

2.4 | Intraperitoneal glucose and insulin tolerance tests (ipGTT and ipITT)

To perform ipGTT, mice were subjected to 10 hr fasting and blood glucose was accessed by tail incision, in the 9th week of treatment. Blood glucose was measured using glucose strips on an Accu-Check Performa II glucometer (Roche, Sao Paulo, Brazil). Glycemic level were measured (0 min) and 15, 30, 60, 90, and 120 min after the administration of glucose (2 g/kg body weight). The AUC of glucose during the ipGTT were calculated. For the ipITT, mice were subjected to 4 hr fasting, and blood glucose was measured before (0 min) and 3, 6, 9, 12, and 15 min after the administration of insulin (0.75 U/kg body weight), in the 10th week of treatment. The kITT (constant rate for glucose disappearance) was calculated as previously described (Akinmokun, Selby, Ramaiya, & Alberti, 1992).

2.5 | Glucose-stimulated insulin secretion in pancreatic islets

The pancreatic islets were isolated by the collagenase method (Boschero et al., 1995), and groups of four islets each were incubated in 0.5 ml of Krebs–Ringer bicarbonate (KRB) buffer (115 mM NaCl, 5 mM KCl, 10 mM NaHCO3, 2.56 mM CaCl2, 1 mM MgCl2, and 15 mM HEPES (Sigma–Aldrich Chemical, St Louis, MO), supplemented with 5.6 mM glucose plus 0.3 % of BSA, and equilibrated with a mixture of 95% O2/5% CO2 to give pH 7.4) for 45 min at 37 °C. Subsequently, these islets were exposed to 1 ml KHBS containing 2.8 mM (low concentration) or 16.7 mM (high concentration) mmol/L glucose for 1 hr at 37 °C. To measured insulin secreted we collected the supernatants that were stored at −20 °C, and the remaining islets were homogenized in an alcohol/acid solution to measure the total insulin content by RIA (Scott et al., 1981).

2.6 | Immunofluorescence

The pancreata were collected and fixed overnight in 4% paraformaldehyde solution at room temperature. Afterward, the dehydration and

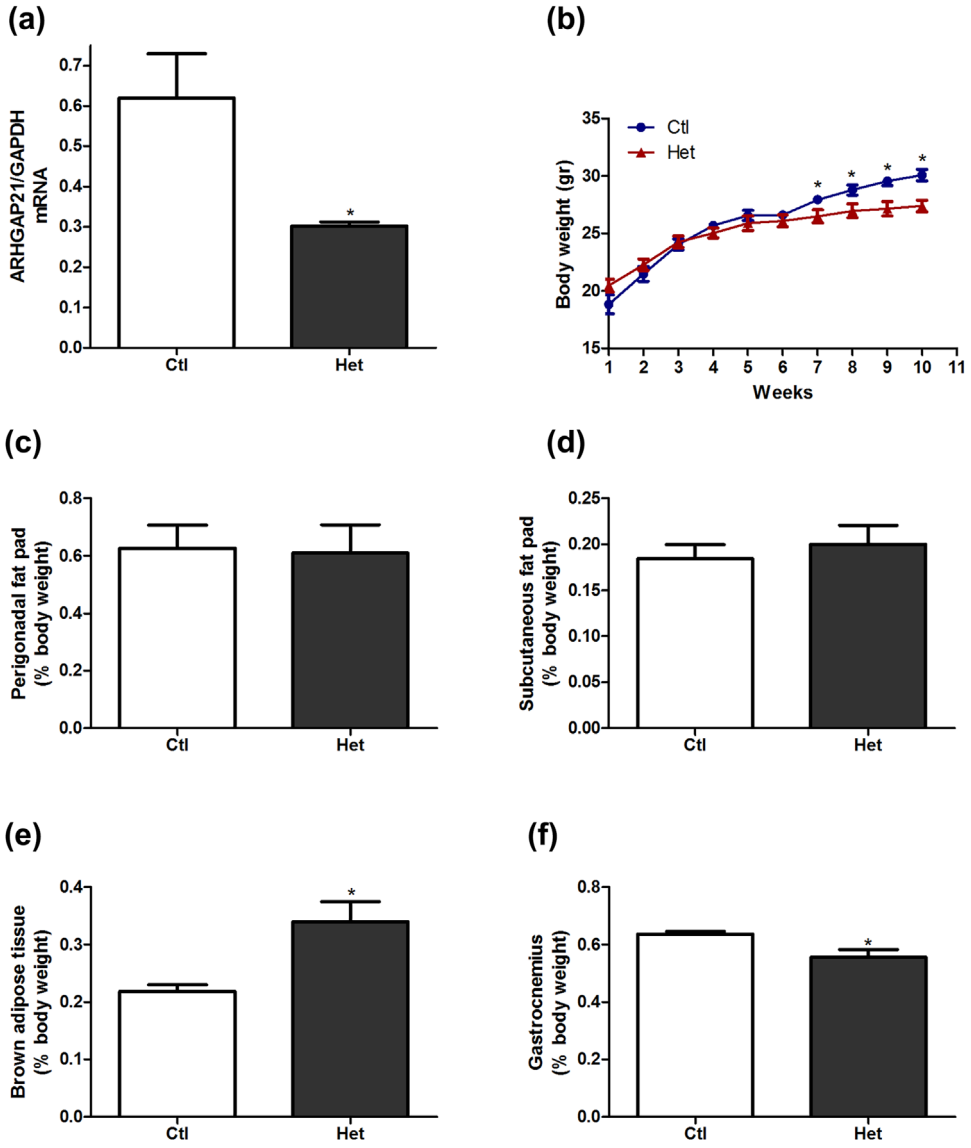

FIGURE 1 Body parameters of ARHGAP21 Het mice. ARHGAP21 expression was evaluated by qPCR (a). Body weight during the experimental period (b). Perigonadal (c) and subcutaneous fat pad (d), brown adipose tissue (e), and gastrocnemius (f) weights. Control mice (Ctl) and ARHGAP21 heterozygous mice (Het) fed a chow diet for 10 weeks. Data are mean ± SEM (n = 5–7). *$p \leq 0.05$ (Student's-t-Test)

impregnation in paraffin was performed and these pancreata were cut into 5-μm-thick sections. The sections were rehydrated, permeabilized, blocked with 0,5% BSA solution and incubated with the primary (Polyclonal Guinea Pig Anti-Insulin, A0564, Dako, Glostrup, Copenhagen, Denmark) and secondary (FITC, F6261, Sigma–Aldrich) antibodies (Barbosa-Sampaio et al., 2015). Finally, the slides were prepared using Mounting Medium for Fluorescence (Dako), and the pancreata were visualized by fluorescence microscopy (Leica CTR 6500, Wetzlar, Alemanha) using 20× objectives. The images were analyzed using the ImageJ software (National Institute of Health, MD).

2.7 | Statistical analysis

To analyze the data we used the Student's t-test (GraphPad Prism 5, La Jolla, CA). The data were presented as means ± standard errors media (SEM), and the difference between the groups were considered statistically significant if $p \leq 0.05$.

3 | RESULTS

3.1 | Body parameters of ARHGAP21 Het mice

First, we characterized the ARHGAP21-haplodeficient mice by quantifying the gene expression of ARHGAP21 and, as expected, Het mice presented approximately 50% less ARHGAP21 than Ctl group (Figure 1a). We measured the body weight of mice once a week for 10 weeks. At the 7th week, Het mice displayed reduced body weight compared with Ctl, and this difference was observed until the end of the experiment (Figure 1b). Although the body weight was reduced, alteration in the weight of perigonadal and

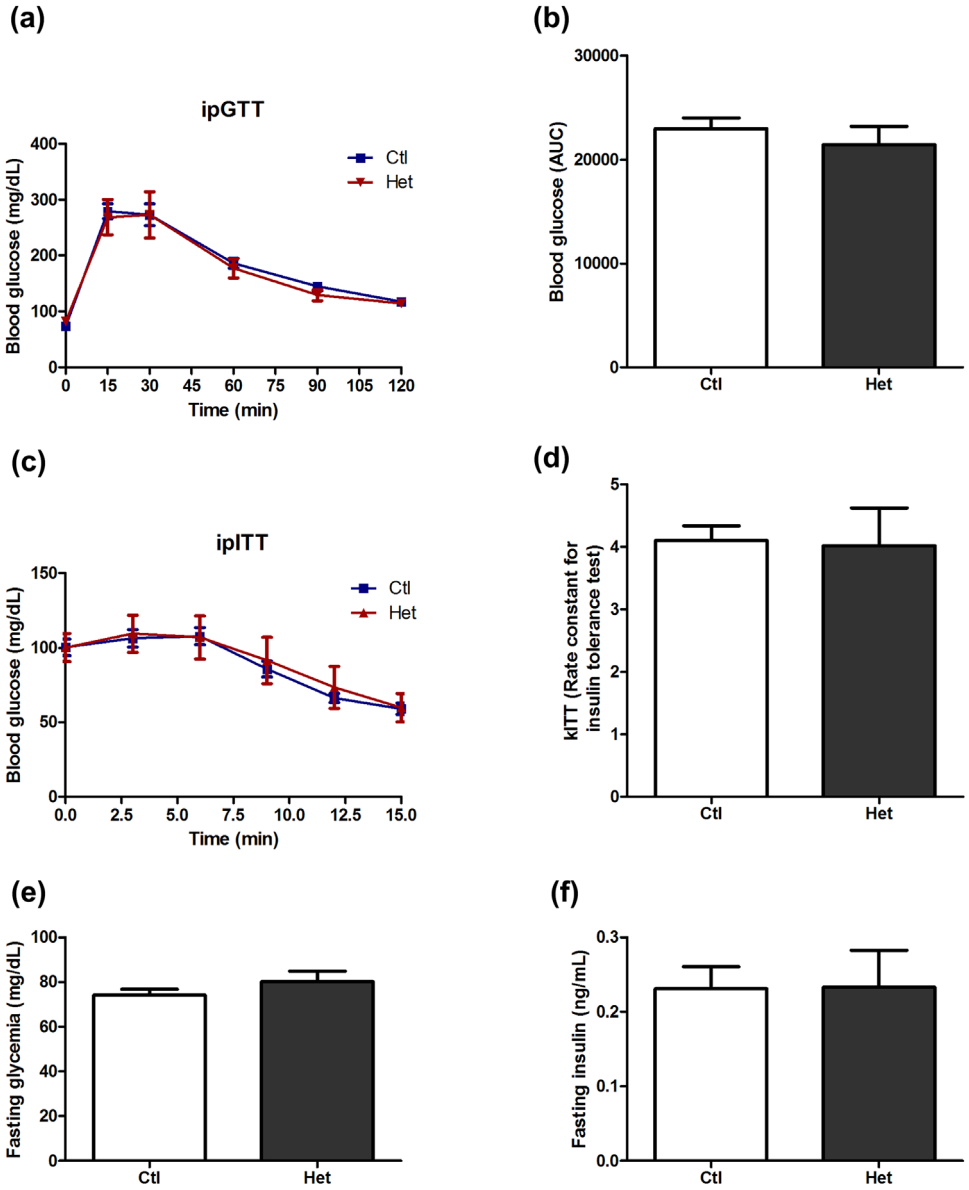

FIGURE 2 Glucose homeostasis of ARHGAP21 Het mice. Changes in blood glucose (a) and AUC of blood glucose (b) during ipGTT. Changes in blood glucose (c) and rate constant for glucose disappearance (kITT) (d) during the ipITT. Fasting glycemia (e) and insulinemia (f). Control mice (Ctl) and ARHGAP21 heterozygous mice (Het) fed a chow diet for 10 weeks. Data are mean ± SEM (n = 5-7). $^*p \leq 0.05$ (Student's-t-Test)

subcutaneous fat pads were not observed in Het mice (Figures 1c and 1d). Interestingly, brown adipose tissue was significantly increased in the Het mice (Figure 1e). In addition, these mice showed a decrease in gastrocnemius skeletal muscle weight (Figure 1f).

3.2 | Glucose homeostasis of ARHGAP21 Het mice

Glucose homeostasis was similar between Het and Ctl groups. Insulin sensitivity, evaluated by ipITT, was similar between Ctl and Het groups (Figures 2c and 2d), which reflected in the same level of glucose tolerance, measured by glucose kinetics after a glucose challenge (ipGTT) (Figures 2a and 2b) as well as the fasted glycemia and insulinemia, which were similar between Ctl and Het groups (Figures 2e and 2f).

3.3 | ARHGAP21 Het-HFD mice displayed reduced body and fat pads weight

We also investigated how the Het mice respond to a high-fat diet challenge. As shown in the Figure 3a, Het-HFD mice displayed a reduction in body weight at 4 weeks, and this was accompanied by a decrease of perigonadal and subcutaneous fat pad weight, compared with Ctl-HFD group (Figures 3b and 3c). Brown adipose tissue was similar between groups (Figure 3d) whereas Het-HFD mice showed a decrease in gastrocnemius skeletal muscle weight (Figure 3e).

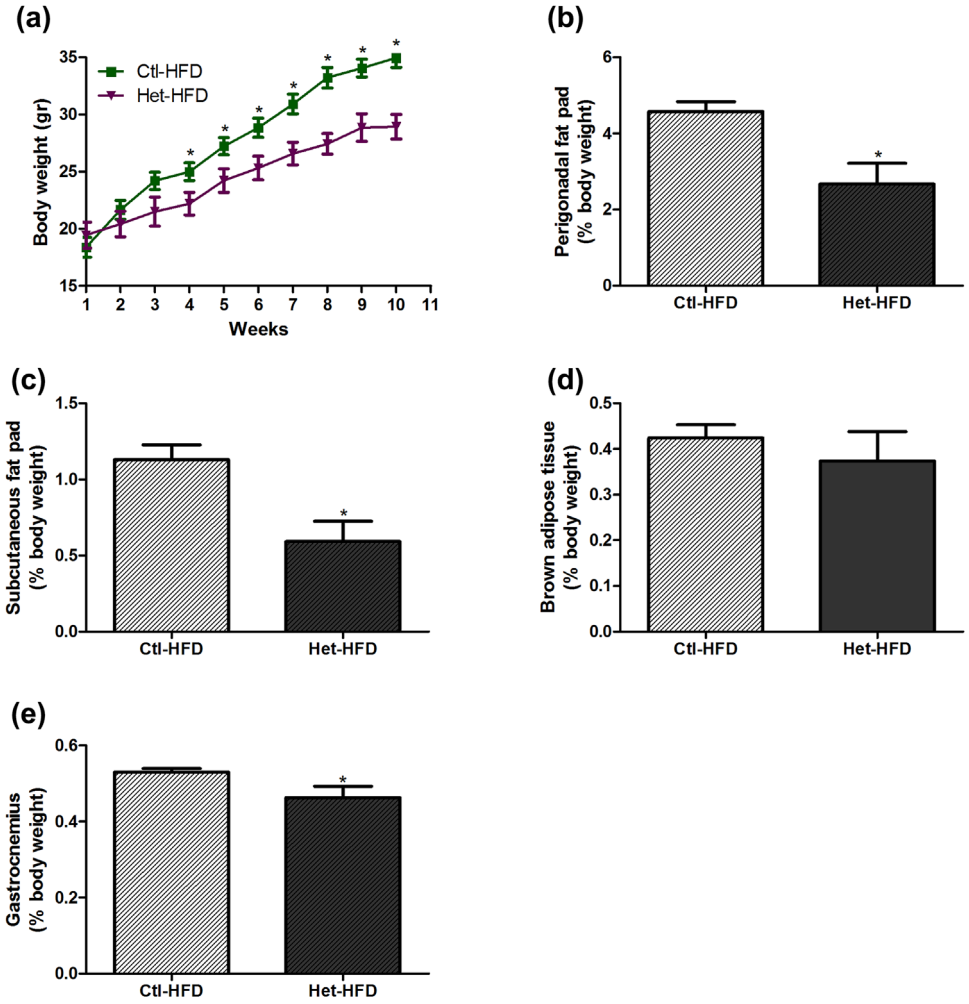

FIGURE 3 ARHGAP21 Het-HFD mice displayed reduced body and fat pads weight. Body weight during the experimental period (a). Perigonadal (b) and subcutaneous fat pad (c), brown adipose tissue (d), and gastrocnemius (e) weights. Control mice (Ctl) and ARHGAP21 heterozygous mice (Het) fed a high-fat diet for 10 weeks. Data are mean ± SEM (n = 4–9). *$p \leq 0.05$ (Student's-t-Test)

3.4 | ARHGAP21 Het-HFD mice showed an improvement in the glucose tolerance and insulin sensitivity

During the ipGTT, blood glucose was reduced at 60, 90, and 120 min (Figure 4a) in the Het-HFD, compared with Ctl-HFD mice, indicating an improvement in glucose tolerance, as confirmed by the AUC (Figure 4b). An improvement in insulin sensitivity also was observed, as judged by reduced blood glucose during ipITT and increased kITT (Figures 4c and 4d), in Het-HFD, compared with Ctl-HFD mice. However, fasting blood glucose was not different between groups (Figure 4e), while lower insulinemia was observed in the Het-HFD, compared with Ctl-HFD group (Figure 4f).

3.5 | ARHGAP21 Het-HFD mice had reduced insulin secretion and beta cell area

To explain the lower insulinemia in the Het-HFD mice we analyzed the glucose stimulated insulin secretion (GSIS), in isolated pancreatic islets. At a high glucose concentration (16.7 mM), we observed reduced insulin secretion in the islets from Het-HFD mice compared with the Ctl-HFD group; whereas the insulin secretion was not different between groups at a low glucose concentration (2.8 mM) (Figure 5a). The total insulin content was reduced in the islets from Het-HFD mice (Figure 5b), and this was associated with a lower beta cell area, compared with the Ctl-HFD mice (Figures 5c and 5d).

4 | DISCUSSION

GAP proteins have specific physiological roles in glucose homeostasis depending on their isoforms (Bos et al., 2007). The inhibition of a Rab-GAP, known as TBC1D1, decreases glucose uptake by skeletal muscle (Chadt et al., 2015; Stöckli et al., 2015; Szekeres et al., 2012), whereas the overexpression of TCGAP, a Rho-GAP protein member, decreases glucose uptake by adipose cells (Chiang et al., 2003). Here, we extend these findings, showing a beneficial effect of whole body reduction of ARHGAP21 in glucose homeostasis, as judged by an improvement in

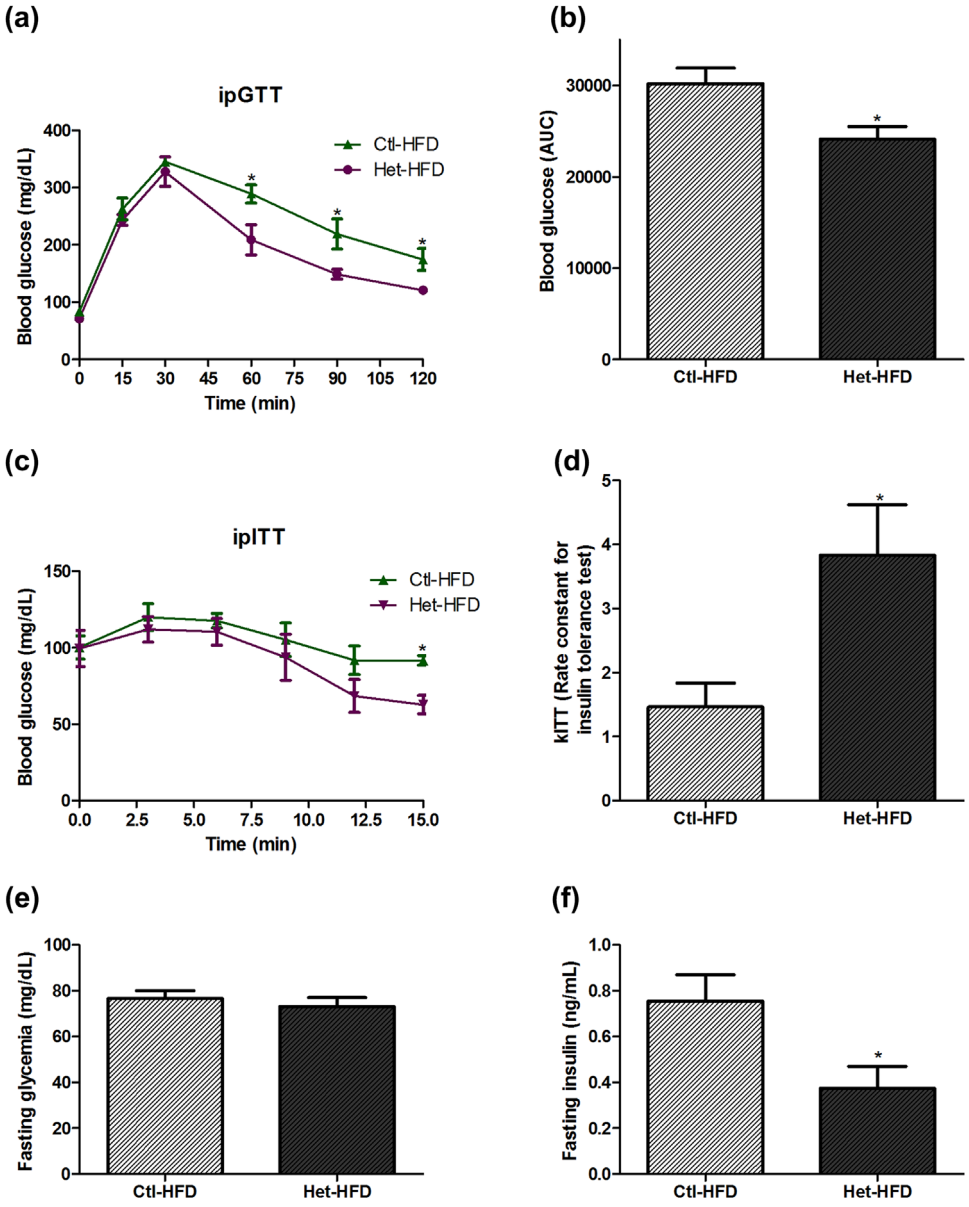

FIGURE 4 ARHGAP21 Het-HFD mice showed an improvement in the glucose tolerance and insulin sensitivity. Changes in blood glucose (a) and AUC of blood glucose (b) during ipGTT. Changes in blood glucose (c) and rate constant for glucose disappearance (kITT) (d) during the ipITT. Fasting glycemia (e) and insulinemia (f). Control mice (Ctl) and ARHGAP21 heterozygous mice (Het) fed a high-fat diet for 10 weeks. Data are mean ± SEM (n = 5–8). *$p \leq 0.05$ (Student's-t-Test)

glucose tolerance and insulin sensitivity, associated with reduced insulin secretion. These effects were observed only in Het-HFD mice.

Our study is the first to show that Rho-GAP ARHGAP21 inhibition increased insulin sensitivity, resulting in glucose homeostasis improvement, suggesting similarities with other Rho-GAP TCGAP that, when overexpressed in adipocytes, impairs glucose uptake (Chiang et al., 2003). The mechanism by which TCGAP impacts the insulin-induced glucose uptake seems to involve a physical interaction with the non-canonical insulin signaling: CAP-Cbl pathway (Baumann et al., 2000; Chiang et al., 2003). As a consequence of insulin stimulation, TCGAP may expose its proline-rich domain, increasing the interaction with CrkII protein (a CAP-Cbl pathway member) (Chiang et al., 2001), habilitating CrkII to carry TCGAP toward the plasma membrane, activating it, and increasing GLUT4 translocation to the cell plasma membrane. However, when these adaptor proteins of the CAP-Cbl pathway are overexpressed, for example, APS protein, a down regulation in the insulin-stimulated glucose uptake occurs, due to an excess of adaptor proteins over endogenous Cbl and insulin receptors, impairing glucose uptake (Chiang et al., 2003; Liu, Kimura, Baumann, & Saltiel, 2002).

As, Rho-GAP ARHGAP21 and TCGAP are proteins with different domains (Bos et al., 2007; Chiang et al., 2003), it is difficult to accept that they display a similar mechanism of action. Nevertheless, more studies are necessary to clarify the molecular mechanism by which

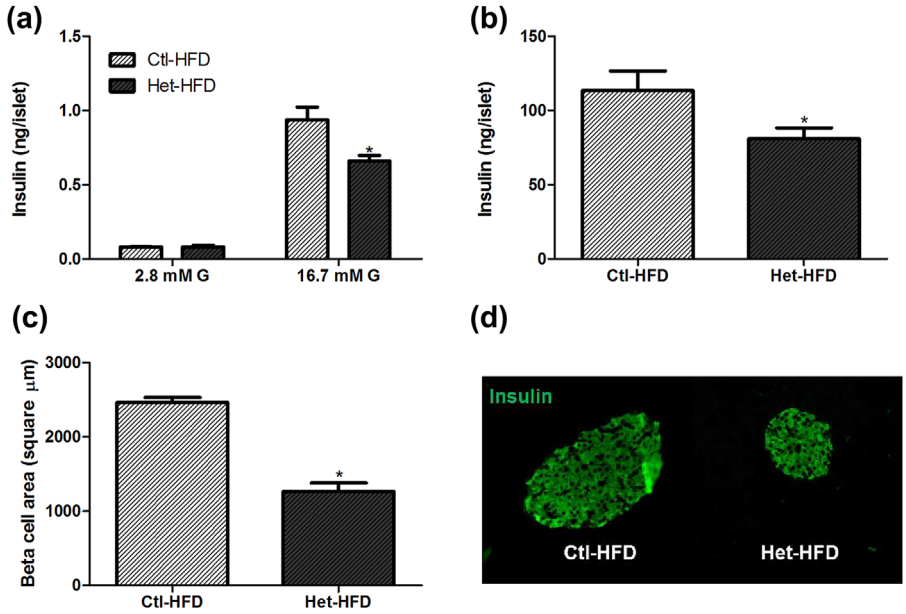

FIGURE 5 ARHGAP21 Het-HFD mice had reduced insulin secretion and beta cell area. Insulin secretion in isolated pancreatic islets stimulated with low (2.8 mM) and high (16.7 mM) glucose concentration (a). Total insulin content of islets (b). Beta cell area (c) and its representative images stained for insulin (d). Images were captured using a 20× objective. Control mice (Ctl) and ARHGAP21 heterozygous mice (Het) fed a high-fat diet for 10 weeks. Data are mean ± SEM (n = 4–8). *$p \leq 0.05$ (Student's-t-Test)

ARHGAP21 inhibition improves peripheral insulin sensitivity, and glucose tolerance.

Probably, as a consequence of improved insulin sensitivity, the Het-HFD mice secrete less insulin and show a smaller pancreatic beta cell area, which helps to explain the lower body weight. Indeed, hyperinsulinemia may increase lipogenesis, as well as reduce fat acids oxidation, potentiating the deleterious effect of obesity (Jung & Choi, 2014; Vázquez-Vela, Torres, & Tovar, 2008).

Another possible explanation for the improvement in glucose homeostasis,observed in the Het-HFD mice, is as a direct effect of ARHGAP21 inhibition upon pancreatic beta cells. In fact, our group demonstrated that its inhibition, specifically in pancreatic islets from neonate mice, increased insulin secretion (Ferreira et al., 2015). However, these experiments were done in immature pancreatic islets, which present different physiological behavior, compared to those explored here (Carvalho et al., 2010; Mendonça, Carneiro, Bosqueiro, Crepaldi-Alves, & Boschero, 1998).

Curiously, the Het mice also show lower body weight. However, this feature occurs by a different mechanism from that observed in Het-HFD mice, as they display reduced muscle weight instead of reduced white adipose tissue, and also an increased brown adipose tissue depot. These results are the first to point to the possible involvement of a Rho-GAP member in the control of body composition, decreasing white adipose tissue in Het-HFD mice, and increasing brown adipose tissue in Het mice. However, further investigation is necessary to figure out the mechanism by which this phenomenon occurs.

In summary, our results support that ARHGAP21 inhibition improves glucose tolerance by ameliorating insulin sensitivity and secretion, when mice are fed on a HFD. In addition, an as-yet undetermined role of a Rho-GAP member, related with body composition, was demonstrated here. Nevertheless, more studies are necessary to clarify the mechanism by which this occurs.

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CONFLICTS OF INTEREST

The authors declared that they have no conflicts of interest.

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REFERENCES

Akinmokun, A., Selby, P. L., Ramaiya, K., & Alberti, K. G. (1992). The short insulin tolerance test for determination of insulin sensitivity: A comparison with the euglycaemic clamp. *Diabetic Medicine*, *9*(5), 432–437.

Barbosa-Sampaio, H. C., Drynda, R., Liu, B., Rodriguez De Ledesma, A. M., Malicet, C., Iovanna, J. L., ... Persaud, S. J. (2015). Reduced nuclear protein 1 expression improves insulin sensitivity and protects against

diet-induced glucose intolerance through up-regulation of heat shock protein 70. *Biochimica et Biophysica Acta*, *1852*(5), 962–969.

Barcellos, K. S., Bigarella, C. L., Wagner, M. V., Vieira, K. P., Lazarini, M., Langford, P. R., . . . Saad, S. T. (2013). ARHGAP21 protein, a new partner of α-tubulin involved in cell-cell adhesion formation and essential for epithelial-mesenchymal transition. *The Journal of Biological Chemistry*, *288*(4), 2179–2189.

Bassères, D. S., Tizzei, E. V., Duarte, A. A., Costa, F. F., & Saad, S. T. (2002). ARHGAP10, a novel human gene coding for a potentially cytoskeletal Rho-GTPase activating protein. *Biochemical and Biophysical Research Communications*, *294*(3), 579–585.

Batista, T. M., Ribeiro, R. A., da Silva, P. M., Camargo, R. L., Lollo, P. C., Boschero, A. C., & Carneiro, E. M. (2013). Taurine supplementation improves liver glucose control in normal protein and malnourished mice fed a high-fat diet. *Molecular Nutrition and Food Research*, *57*(3), 423–434.

Baumann, C. A., Ribon, V., Kanzaki, M., Thurmond, D. C., Mora, S., Shigematsu, S., . . . Saltiel, A. R. (2000). CAP defines a second signalling pathway required for insulin-stimulated glucose transport. *Nature*, *407*(6801), 202–207.

Bigarella, C. L., Borges, L., Costa, F. F., & Saad, S. T. (2009). ARHGAP21 modulates FAK activity and impairs glioblastoma cell migration. *Biochimica et Biophysica Acta*, *1793*(5), 806–816.

Bigarella, C. L., Ferro, K. P., Barcellos, K. S., Martins-de-Souza, D., Traina, F., Novello, J. C., . . . Archangelo, L. F. (2012). Post-translational modification of the RhoGTPase activating protein 21, ARHGAP21, by SUMO2/3. *FEBS Letters*, *586*(19), 3522–3528.

Bishop, A. L., & Hall, A. (2000). Rho GTPases and their effector proteins. *Biochemical Journal*, *348*(Pt 2), 241–255.

Bos, J. L., Rehmann, H., & Wittinghofer, A. (2007). GEFs and GAPs: Critical elements in the control of small G proteins. *Cell*, *129*(5), 865–877.

Boschero, A. C., Szpak-Glasman, M., Carneiro, E. M., Bordin, S., Paul, I., Rojas, E., & Atwater, I. (1995). Oxotremorine-m potentiation of glucose-induced insulin release from rat islets involves M3 muscarinic receptors. *American Journal of Physiology*, *268*((2 Pt 1)), E336–E342.

Carvalho, C. P., Barbosa, H. C., Britan, A., Santos-Silva, J. C., Boschero, A. C., Meda, P., & Collares-Buzato, C. B. (2010). Beta cell coupling and connexin expression change during the functional maturation of rat pancreatic islets. *Diabetologia*, *53*(7), 1428–1437.

Chadt, A., Immisch, A., de Wendt, C., Springer, C., Zhou, Z., Stermann, T., . . . Al-Hasani, H. (2015). "Deletion of both Rab-GTPase-activating proteins TBC1D1 and TBC1D4 in mice eliminates insulin- and AICAR-stimulated glucose transport [corrected]. *Diabetes*, *64*(3), 746–759.

Chiang, S. H., Baumann, C. A., Kanzaki, M., Thurmond, D. C., Watson, R. T., Neudauer, C. L., . . . Saltiel, A. R. (2001). Insulin-stimulated GLUT4 translocation requires the CAP-dependent activation of TC10. *Nature*, *410*(6831), 944–948.

Chiang, S. H., Hwang, J., Legendre, M., Zhang, M., Kimura, A., & Saltiel, A. R. (2003). TCGAP, a multidomain Rho GTPase-activating protein involved in insulin-stimulated glucose transport. *EMBO Journal*, *22*(11), 2679–2691.

Ferreira, S. M., Santos, G. J., Rezende, L. F., Gonçalves, L. M., Santos-Silva, J. C., Bigarella, C. L., . . . Barbosa-Sampaio, H. C. (2015). ARHGAP21 prevents abnormal insulin release through actin rearrangement in pancreatic islets from neonatal mice. *Life Sciences*, *127*, 53–58.

Hall, A. (1998). Rho GTPases and the actin cytoskeleton. *Science*, *279*(5350), 509–514.

Jung, U. J., & Choi, M. S. (2014). Obesity and its metabolic complications: The role of adipokines and the relationship between obesity, inflammation, insulin resistance, dyslipidemia and nonalcoholic fatty liver disease. *International Journal of Molecular Sciences*, *15*(4), 6184–6223.

Kowluru, A. (2010). Small G proteins in islet beta-cell function. *Endocrine Reviews*, *31*(1), 52–78.

Lamarche, N., & Hall, A. (1994). GAPs for rho-related GTPases. *Trends in Genetics*, *10*(12), 436–440.

Liu, J., Kimura, A., Baumann, C. A., & Saltiel, A. R. (2002). APS facilitates c-Cbl tyrosine phosphorylation and GLUT4 translocation in response to insulin in 3T3-L1 adipocytes. *Molecular and Cellular Biology*, *22*(11), 3599–3609.

Luo, N., Guo, J., Chen, L., Yang, W., Qu, X., & Cheng, Z. (2016). ARHGAP10, downregulated in ovarian cancer, suppresses tumorigenicity of ovarian cancer cells. *Cell Death & Disease*, *7*, e2157.

Mendonça, A. C., Carneiro, E. M., Bosqueiro, J. R., Crepaldi-Alves, S. C., & Boschero, A. C. (1998). Development of the insulin secretion mechanism in fetal and neonatal rat pancreatic B-cells: Response to glucose, K+, theophylline, and carbamylcholine. *Brazilian Journal of Medical and Biological Research*, *31*(6), 841–846.

Perillo, L., Monsurrò, A., Bonci, E., Torella, A., Mutarelli, M., & Nigro, V. (2015). Genetic association of ARHGAP21 gene variant with mandibular prognathism. *Journal of Dental Research*, *94*(4), 569–576.

Rütti, S., Arous, C., Nica, A. C., Kanzaki, M., Halban, P. A., & Bouzakri, K. (2014). Expression, phosphorylation and function of the Rab-GTPase activating protein TBC1D1 in pancreatic beta-cells. *FEBS Letters*, *588*(1), 15–20.

Scott, A. M., Atwater, I., & Rojas, E. (1981). A method for the simultaneous measurement of insulin release and B cell membrane potential in single mouse islets of Langerhans. *Diabetologia*, *21*(5), 470–475.

Sousa, S., Cabanes, D., Archambaud, C., Colland, F., Lemichez, E., Popoff, M., . . . Cossart, P. (2005). ARHGAP10 is necessary for alpha-catenin recruitment at adherens junctions and for Listeria invasion. *Nature Cell Biology*, *7*(10), 954–960.

Stöckli, J., Meoli, C. C., Hoffman, N. J., Fazakerley, D. J., Pant, H., Cleasby, M. E., . . . James, D. E. (2015). The RabGAP TBC1D1 plays a central role in exercise-regulated glucose metabolism in skeletal muscle. *Diabetes*, *64*(6), 1914–1922.

Szekeres, F., Chadt, A., Tom, R. Z., Deshmukh, A. S., Chibalin, A. V., Björnholm, M., . . . Zierath, J. R. (2012). The Rab-GTPase-activating protein TBC1D1 regulates skeletal muscle glucose metabolism. *American Journal of Physiology Endocrinology and Metabolism*, *303*(4), E524–E533.

Van Aelst, L., & D'Souza-Schorey, C. (1997). Rho GTPases and signaling networks. *Genes & Development*, *11*(18), 2295–2322.

Vázquez-Vela, M. E., Torres, N., & Tovar, A. R. (2008). White adipose tissue as endocrine organ and its role in obesity. *Archives of Medical Research*, *39*(8), 715–728.

Wennerberg, K., Rossman, K. L., & Der, C. J. (2005). The Ras superfamily at a glance. *Journal of Cell Science*, *118*(Pt 5), 843–846.

Xavier-Ferrucio, J., Ricon, L., Vieira, K., Longhini, A. L., Lazarini, M., Bigarella, C. L., . . . Saad, S. T. O. (2017). Hematopoietic defects in response to reduced Arhgap21. *Stem Cell Research*, *26*, 17–27.

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