Hyperglycemia and hyperinsulinemia stimulate cervical cancer cell proliferation *in vitro* and *in vivo* study

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Abstract

Background: Diabetes mellitus and malignant tumor are the second and third causes of women death in Mexico. Hyperglycemia, insulin and insulin-like growth factor 1 are the main risk factors involved in cancer development in patient with diabetes. The aim of this study was to evaluate the effect of hyperglycemia and hyperinsulinemia over cell proliferation and tumor growth in cervical cancer.

Methods: Cell proliferation, apoptosis and cell cycle of cervical cancer cell lines (HeLa, SiHa and CaSki) in presence of hyperglycemia and/or insulin were evaluated. Xenograft model for cervical cancer was done in diabetic female nu/nu mice; biochemical parameters, body weight, tumoral volume and cell doubling time were evaluated.

Results: Hyperglycemia and hyperinsulinemia significantly increase cell proliferation and decreases apoptosis with no change in cell cycle. Insulin treatment increase tumor volume and diminish cell doubling time, this group also developed hyperinsulinemia and in Langerhans pancreatic islet hypertrophy; whereas, hyperglycemic groups show the same effects but in lesser degree than the insulin treated group.

Conclusion: Glucose and insulin stimulates both, proliferation and tumoral growth of cervical cancer, so this should be a possible explanation for the low survival of diabetic patients with cervical cancer in compare to non-diabetic patients with cervical cancer.

Background

Diabetes mellitus is a metabolic alteration of carbohydrates, lipids and proteins associated to insulin deficiency or resistance, that prevents correct glucose metabolism causing increased glucose levels [1]. King and coworkers [2], estimate that by the year 2025, diabetes prevalence will increase 27%. By the way, Wild and coworkers [3], estimated that for the year 2030 diabetic people will be duplicated in the most of countries of world, without consider obesity. Those estimations are very worrying because projections increase mortality and decrease quality of life. In Mexico, diabetes and malignant tumors represent the second and third cause of death respectively in both women and men [4]. Cervical cancer continues being the second cause of death in women by this disease, despite free programs of early detection of cervical cancer. These dates are alarming because the costs of treatments of both diseases are expensive for people and National Health Institutes, and the combination of both diabetes and cancer diminish cancer survive.

It has been pointed out an association between diabetes and cancer, there are relative risk associated with diabetes for hepatic, pancreatic, endometrial, colon, breast, bladder and lung cancer, although the relative risk varies between each type of cancer [5]; unfortunately, in our country the probability of having cancer and diabetes is becoming more frequent. Reportedly that patient with both pathologies: cancer and diabetes does not respond equal to cancer treatment and have higher mortality comparing with no diabetic patient [6, 7]. A lot of studies have reported that growth factors increase metastasis and invasion

properties of cancer cells (epidermal growth factor (EGF) and Insulin-like growth factor 1 (IGF-1)) [8–11]. In normal condition, in smooth muscle cells in presence of IGF-1 promotes protein synthesis but no cell proliferation, conversely, if the same smooth muscle cell culture with some stress condition as hyperglycemic and/or hypercholesterolemic, IGF-1 stimulates cellular proliferation [12, 13]. On the other hand, insulin is responsible for introduce glucose into the cell and subsequently be metabolized; both receptors IGF-1 and insulin belong to tyrosine kinase transmembrane family; this similarity allow that insulin can bind to IGF-1 receptor (IGF-1R) and also IGF-1 can bind to insulin receptor (IR), but with low affinity [14]. Therefore, it is possible that in cancer patients who already have diabetes and are treated with insulin, this insulin could have two effects: introduce glucose into the cancer cell or act as grow factor binding to IGF-1R and promoting proliferation, migration and invasion. IGF-1R is overexpressed in many cancer cell lines and types of tumors, including cervical cancer [11] so is probable that IGF-IR is activated in presence of insulin [15]. Therefore, our objective was to test if insulin may act as a growth factor in promoting tumoral growth. To evaluate the effect of high concentrations of glycemic and insulin over proliferation of cervical cancer cells, we developed the following experiments in vitro and in vivo.

Methods

HeLa, SiHa and CaSki cervical cells were obtained from American Type Culture Collection (ATCC), cells were maintained in Dulbecco's Modified Eagle´s Medium (DMEM) supplemented with 5% of fetal bovine serum (FBS), 37 ^{o}C and 5% of CO_2. HeLa cell line was also used in the "*in vivo"* studies. All experiments were prepared under sterility into a laminar flow hood. All compounds used were purchased from recognized commercial houses.

Effect of insulin on cell proliferation

Cell proliferation was evaluated by MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; Sigma) and crystal violet assays. $1X10^3$ cells/well was seed and expose with normoglycemic (5 mM) or hyperglycemic (25 mM) medium by 7 days alone or in combination with insulin (0, 0.01, 0.1 or 1 ug/mL). SiHa and CaSki cells were only expose to the highest concentration of insulin. All experiments were made by triplicate. Time of exposition was stablished as a result of previous experiments made in the laboratory and by reports in the literature using different cancer cell line. Insulin concentration was selected because high physiological levels has been reported as inductors of proliferative effects (up 10 nM) [16, 17].

MTT Assay

The assay is based on the conversion of MTT to formazan, this compound is developed by viable cells with active metabolism. The measured absorbance is proportional to the number of viable cells. Briefly, 5 mg/mL MTT was dissolved in DMEM (1:9), the solution was added in each well and was incubated during 1 to 3 h. Formazan dye was dissolved with 2-propanol acid, optical density was measured using

an ELISA reader at λ= 570 nm. The results were expressed as the percentage of MTT reduction in relation to cero insulin concentration groups.

Apoptosis assay

Apoptosis was evaluated by flow cytometry. Briefly, once the cells were expose to hyperglycemia and/or insulin, they were washed with PBS and harvested with PBS-EDTA-Tripsin, followed by centrifugation and resuspended in DMEM, finally they were exposed to Guava Nexin Reagent, in the dark at room temperature for 20 minutes. 10,000 events were acquired in the Guava easyCyte equipment (Merck Millipore, Darmstadt, DE) per sample. Early and late apoptosis were determined, and the results were expressed as the total percentage of apoptosis. Experiments were carried out independently and by triplicate.

Cell cycle assay

Determination of cell cycle was performed by flow cytometry. Briefly, once the cells were expose whit hyperglycemia and/or insulin, the cells were washed with PBS and harvested with PBS-EDTA-Tripsin, followed by a centrifugation. After that were fixed in 70% ethanol and incubate at 4 ^{o}C for 24 h. Thereafter, cells were washed with PBS and incubated with Guava Cell Cycle Reagent at room temperature for 30 minutes in the dark. 10,000 events were acquired in the cellCycle software (Merck Millipore, Darmstadt, DE) per sample and cell cycle phases were analyzed. Experiments were carried out independently and by triplicate.

In vivo experiments

Female mice BALB/c (nu/nu) of 20-25 g body weight were purchased from laboratory animal production and experimentation unit (UPEAL-Bioterio) of Universidad Autonoma Metropolitana Unidad Xochimico. The mice had free access to sterile water and food, they were housing into microisolator (Allentown Inc.) under 25^{o}C, 70% of humidity and 12 h light-12 h dark cycle. The sample size was calculate by the comparison of two means, where the sample size corresponds to the value of the standard deviation, with Zα (Z of alpha) which refers to the type I error (confidence level α = 0.05 corresponding to a value of Z = 1.96), and Zβ (beta Z) with a power of 80% (value of Z = 0.84) [18]. All the mice were handling in accord to Mexican Guide (NOM-062-ZOO-1999) and the Committee for the Update of the Guide for the Care and Use of Laboratory Animals and Biological hazard residues Guide (NOM-087-ECOL-SSA1-2001) [19-21]. This protocol was approved for the Instituto Nacional de Cancerología (011/045/IBI and CB714/11).

Experimental Design

Thirty-nine female mice (BALB/c (nu/nu) of 20-25 g body weight) were used. Random way, one part of the mice was hyperglycemia induced using streptozotocin (100 mg/Kg/week) until the mice became hyperglycemic. Once the mice present hyperglycemia (≥ 125 mg/dL), then were inoculated

subcutaneously into the back with $5X10^6$ HeLa cervical cancer cells. Once the tumor reached around 170 mm^3 (week 4), mice were divided randomly in four groups: A) control (no hyperglycemic), B) insulin (1IU/Kg i.p. daily until the end of experiment, no hyperglycemic), C) hyperglycemia and D) hyperglycemia+insulin. The animals were weekly weight, and glucose blood concentration as well as tumor size were measured. The tumor xenografts were measured with a caliper in two dimensions; tumor volume was calculated by following equation: $V=((width)^2*length*\pi)/4)$ [22]; time of duplicated rate of tumor was calculate using the following equation: $T=((days\ of\ treatment)/(((Log_{(final\ volume)}-Log_{(initial\ volume)})/Log\ 2))$ [23]. When the tumor reaches 2000 mm^3, the mouse was sacrificed. At the end of experiments the mice were sacrificed under anesthesia overdoses using a mixture of isoflurane/oxygen (3%); plasma and pancreas were obtained; samples were store in formaldehyde at 10% for histological studies.

Biochemical quantifications

Glucose, cholesterol, triglycerides, creatinine and BUN were quantified in serum at the end of the experiments using a Beckman Coulter laboratory analyzer AU680 Chemistry System. Also, blood glucose concentration was measured with glucometer (Accu-Chek Performa, Roche) each week for hyperglycemia follow-up. Plasma insulin concentration was determined using an ELISA kit (Abnova).

Histological studies

Pancreas was perfused through pancreatic conduct catheter with saline solution following by 10% formalin until fixation was completed. After that, the tissue was including in paraffin, sectioned at 3 µm thickness and stained with hematoxylin-eosin method. The slides were analyzed blindly. Langerhans islets by mm^2 were count in all groups.

Statistics analyses

All the results were present as mean ± SEM (standard error of the mean). The tumor volume was analyzed by ANOVA for repeated measures followed by the Student Newman-Keuls comparison test. Overall survival was calculated with Kaplan-Meier analysis. The other parameters were evaluated using one-way ANOVA followed by Student Newman-Keuls multiple comparison test, GraphPad Prism 4 software (San Diego, CA, USA) and SPSSv.21.0.0 (Chicago, IL, USA) were used. Statistical significance was defined when p value was ≤ 0.05.

Results

To test our hypothesis, we first perform in vitro assays; we quantified percentage of cell proliferation with high glucose concentration (25 mM) with or without insulin (Table 1), we found that in Hela cells under hyperglycemia condition, glucose lead to significant increase of cell proliferation (12%) in compare to normoglycemic condition. Those results were observed with both MTT and crystal violet assay. After that, the cells were expose to different insulin concentration (0.01, 0.1 and 1 µg/mL); in Table 1 we show that

in normoglycemic condition (glucose 5 mM), insulin did not modify cell proliferation, conversely, in hyperglycemic condition with high insulin concentration (1 µg /mL) this increased cell proliferation, more that 20%.

Table 1
Hyperglycemia and insulin effect over cell viability (%) in Hela cells.

Insulin [ug/mL]	Glucose [5mM]	Glucose [25mM]
0.00	100.0 ± 4.7	112.3 ± 3.7 [a]
0.01	92.1 ± 2.3	115.4 ± 4.3 [a]
0.10	95.0 ± 3.4	118.5 ± 4.2 [a]
1.00	103.6 ± 3.3	126.2 ± 3.3 [a,b]

Each experiment was the average of three independent experiments. a $P \leq 0.05$ vs glucose 5 mM without insulin; b $P \leq 0.05$ vs glucose 25 mM without insulin.

We also evaluated the effect of cell proliferation in SiHa and CaSki cell lines in hyperglycemia and hyperinsulinemia condition. In the Table 2, we showed that in both cell lines had a significant increase on cell proliferation in presence of hyperglycemia and this proliferation show a tendency to increases even more when insulin (1 µg /mL) was added.

Table 2
Hyperglycemia and hyperinsulinemia effect over cell viability percentage in SiHa and CaSki cell lines.

	Normoglycemia	Hyperglycemia	Hiperglicemia + Insulin
SiHa	100 ± 3.5	128.6 ± 5.5 [a]	145.0 ± 12.4 [a]
CaSki	100 ± 6.0	127.7 ± 5.0 [a]	136.3 ± 10.2 [a]

Each experiment was the average of three independent experiments. a $P \leq 0.05$ vs Normoglycemia without insulin. Results are expressed as mean ± SEM.

To elucidate a possible mechanism of action, we explored the apoptosis and cell cycle in the three cervical cancer cell lines. In Fig. 1 we show apoptosis percentage in Siha and CaSki cells. Clearly, we observed a decrease in the total percentage of apoptosis in the hyperglycemia group in compared to the control group with statistic significance. When insulin was added, this percentage decreases even more, although there was not significant difference respect to only hyperglycemia group. In the other hand, in HeLa cell line we did not find statistical difference when insulin was added, only we find a decreasing tendency. Whereas, any of three cells lines show change in cell cycle phases when the cells were treated with insulin or hyperglycemia (data no show).

Once we found that both conditions hyperglycemia and hyperinsulinemia promote the cell proliferation; we translate these assays to in vivo studies in order to observe if the results obtained in vitro are reproduced in vivo. Blood glucose levels were monitored during the twelve weeks, Fig. 2B shows the temporal course of blood glucose levels where it is clearly observed that animals administered with streptozotocin significantly increased serum glucose with respect to the control group. Systemic toxicity was evaluated recording body weight during all experiment (12 weeks), no difference was found in this parameter (Fig. 2A).

Several biochemical parameters were quantified in order to know if the hyperglycemic status developed by the mice already have the metabolic alterations that occurs during diabetes in humans. Figure 2C shows the levels in plasma of glucose, cholesterol, triglycerides, creatinine and BUN in mice of all groups studied measured at the end of experiment. Hyperglycemic mice show significative increases of all parameters and insulin administration was only able to prevent triglycerides increase.

When islets of Langerhans were analyzed, we found changes in the number of islets per square millimeter, as well as the insulin serum levels. In Fig. 3B, we show a decrease in the number of Langerhans islets in all experimental groups although only hyperglycemic group reached significant statistical difference. Additionally, the group administrated with insulin show a slight hypertrophy; in fact, mice administered with streptozotocin show inflammatory cells. When fasting insulin levels were quantified, insulin group shows an increase of insulin levels in compare with the control group, while hyperglycemic groups not show any change in compare with the control group, even one of the hyperglycemic groups was administered with insulin (Fig. 3C). Those results indicate that insulin doses administrated caused important physiological changes without controlling circulating glucose levels.

In Fig. 4A we show a temporary course of tumoral volume of xenotransplants of HeLa cells. We found a significantly increases of tumor growth in all studied groups in comparison to the control group; in fact, insulin group, independently of hyperglycemia condition, shows the highest tumor growth, some mice of this group had to be sacrificed before to finish the treatment because of ethic guidelines does not allow us to work with such big tumor (Fig. 4B). We did not find significant changes in the tumoral volume in hyperglycemia groups with or without insulin, however both groups show an increase in tumoral volume, that can suggest that both insulin and glucose are activating signaling pathways that culminate in cell proliferation and/or decreases in apoptosis.

Those results correlate with a decrease of cell doubling time, while control group need approximately 20 days to duplicate the tumoral volume, insulin´s groups need only approximately 13.5 days (Fig. 4C).

Discussion

Current evidence demonstrates that hyperglycemia promote cell proliferation to triggers several direct and indirect pathways such as induction of epithelial mesenchymal transitions [24], high density in GLUT 1 receptors and used of lactate [25, 26], increase leptin and pro-survival AKT/mTOR signaling [27], increase levels of inflammatory cytokines in blood circulation [28, 29] and WNT/β-catenin signaling [30]. These

studies suggest that high glucose levels have an influence in clinical course of cancer patients. In this study we have demonstrated in vitro that the combination of hyperglycemia and hyperinsulinemia act synergistically to enhance cell proliferation in cervical cancer cells and decrease apoptosis without cell cycle changes.

Those results are similar to report by Masur and coworkers [31], they report that glucose and insulin increase proliferation cell and migratory activity in colon, mama, bladder and prostate cell lines through activation of phosphatidylinositol 3-kinase (PI3K), protein kinase C alpha (PKCα) and human myosin light chain kinase (MLCK) proteins. However, in our study we did no find significant changes in the cell cycle, similar results were reported by Liu and coworkers in head and neck cells lines [30], unlike Masur et. al. [31] who found an increase number of transcripts of cyclin A1 and E, genes involved in the transition from G1 to S phase, nevertheless, they do not confirm these results with a whole cell, so additional assay will be done.

Both insulin and IGF-1 have similar structure and activate almost the same transduction pathway; IR has metabolic function, when insulin activate IR, adapter proteins that contain phosphor-tyrosine binding as insulin receptor substrate (IRS-1-4) are phosphorylated, this initiates an autocatalytic process that culminates with the activation of proteins that allow translocations of GLUT4 receptors to cellular membrane for transport of glucose inside the cell. While, IGF-1R has proliferation, grow and anti-apoptosis effects; once IGF-1R is activated by IGF-1, IRS and Shc (Src homology collagen) are phosphorylated, which subsequently interact with p85 and p110 subunit of PI3K protein who activated AKT and in turn promotes cell growth and protein synthesis. Both insulin and IGF-1 can bind of both of two receptors IR and IGF-1R (although their affinity to own receptor is higher). It has been reported that in pathological conditions with hyperinsulinemia, insulin can trigger proliferation pathway activated by IGF-1 in mama, colon, cervix, kidney and lung cancer cells lines [32–37]. In addition, IR and IGF-1R are closely linked stimulate cellular proliferation and induce metastasis in experimental models [29, 38]. In this study we found that, in vitro, both hyperinsulinemia and hyperglycemia increase cell proliferation and decrease apoptosis, whereas in vivo increases tumoral volume. So, our results are related to what is reported in the literature.

By the way, Novosyadlyy et. al. [39] performed in vivo studies, where shows that high phosphorylation of insulin and IGF-1 receptors in mammalian tumor cell extracted from diabetic rats, suggesting that hyperinsulinemia also have an influence on IGF-1 pathway. Our study shows that in normoglycemic condition there are no changes in presences of insulin (Table 1), wherever, insulin plus hyperglycemia increases cell proliferation significantly (more than 25%), and the same effect was observed in cisplatin resistant cells (Table 2). Even though that change seems not so big, it is significant and enough to increases tumoral volume (Fig. 4A) and cell doubling time (Fig. 4B). Similar results were show in smooth muscle cells (SMC), by Clemmons and coworkers [40]. These authors observed that the IGF-1 stimulates both migration and proliferation in SMC in presence of hyperglycemia through activation αVβ3 integrin and Shc phosphorylation. It is worth mentioning that IGF-1R is present in cervical cancer cell line [11].

In this study we only used insulin but not IGF-1, and the group administered with insulin showed an increase in serum insulin levels (Fig. 3C) and also, this result correlated with the increase in tumor volume (Fig. 4A), indirectly indicating the possible activation of the IGF-1 pathway. However, the diabetic groups treated or not with insulin, also showed an increase in tumor volume although the serum insulin levels were the same to the control group, which suggests that the effect is due to hyperglycemia condition more than insulin. According to these results, Villarreal-Garza and coworkers [41] reported that patients with breast cancer and hyperglycemia are associated with a poor outcome in contrast to normoglycemic patients, conferring an elevated risk to die. This effect is probably due to the low chemotherapy response produced by hyperglycemia when induced high proliferation, so it will be necessary corroborate it.

Our results show an increase of insulin concentration in insulin treated group (Fig. 3C) and that result correlated with a hypertrophy in the Langerhans islets (Fig. 3A). This effect supports the results reported by Gao and coworkers [42] finding that a high-fat diet impaired glucose homeostasis increasing insulin release and causing hypertrophy in the Langerhans islets, which in turn resulted in insulin resistance.

Insulin stimulates both, proliferation and tumoral growth of cervical cancer, and this could be one of the possible causes for what diabetic people with cervical cancer present a low response to chemotherapy in compare to normoglycemic patients. This propose is similar that reported by the Liu group [30], where they found that the long cultivation with high-glucose environment promote that the head and neck cancer increase malignancy, regardless of the lymph node metastasis that occurs in this type of cancer.

Conclusion

The present study confirms that both hyperglycemia and hyperinsulinemia enhance cell proliferation and tumor growth in cervical cancer. So, clinicians must be permanently monitoring glucose levels to avoid hyperglycemia or hyperinsulinemia in decrement of cancer response.

Abbreviations

DMEM Dulbecco’s Modified Eagle´s Medium

EGF Epidermal growth factor

FBS Fetal bovine serum

IGF-1 Insulin-like growth factor 1

IGF-1R Insulin-like growth factor 1 receptor

IR Insulin receptor

ISR Insulin receptor substrate

MTT 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium

MLCK Myosin Light Chain Kinase

PKCα Protein kinase C alpha

PI3K Phosphatidylinositol 3-kinase

Declarations

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Author contributions

MGG performed the cell cultures and data analysis under the supervision of JPR, IFM performed part of the animal's experiments and data analysis under the supervision of JPR, RJ performed the cell cultures and reviewed the manuscript, NU performed the histological experiments and analyzed the data, PGL contributed reagents, materials and supervised the study and JPR designed experiments, interpreted the data and wrote the manuscript.

Competing interest: The authors declare no competing interest.

Ethics approval and consent to participate: The study was in approved by the Committee of Instituto Nacional de Cancerología and was performed for the national guide (NOM-062-ZOO-1999) and Committee for the Update of the Guide for the Care and Use of Laboratory Animals.

Consent: Not Applicable.

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Availability of data and materials: All the data generated or analyzed in this study are included in the manuscript or the supplementary information.

References

1. NORMA Oficial Mexicana NOM-015-SSA2-1994, Para la prevención, tratamiento y control de la diabetes mellitus en la atención primaria. http://www.salud.gob.mx/unidades/cdi/nom/015ssa24.html.

2. King H, Aubert RE, Herman WH. Global burden of diabetes, 1995-2025: prevalence, numerical estimates, and projections. Diabetes Care. 1998;21(9):1414-31. doi:10.2337/diacare.21.9.1414
3. Wild S, Roglic G, Green A, Sicree R, King H. Global prevalence of diabetes: estimates for the year 2000 and projections for 2030. Diabetes Care. 2004;27(5):1047-53. doi:10.2337/diacare.27.5.1047
4. Estadísticas vitales, 2010. Registros de Mortalidad INEGI. www.inegi.org.mx/saladeprensa/aproposito/2017/muertos2017_Nal.pdf
5. Szablewski L. Diabetes mellitus: influences on cancer risk. Diabetes Metab Res Rev. 2014;30(7):543-53. doi:10.1002/dmrr.2573
6. Zendehdel K, Nyrén O, Ostenson CG, Adami HO, Ekbom A, Ye W. Cancer incidence in patients with type 1 diabetes mellitus: a population-based cohort study in Sweden. J Natl Cancer Inst. 2003;95(23):1797-800. doi:10.1093/jnci/djg105
7. Jiménez-Garcia R, Hernandez-Barrera V, Carrasco-Garrido P, Gil A. Prevalence and predictors of breast and cervical cancer screening among Spanish women with diabetes. Diabetes Care. 2009;32(8):1470-2. doi:10.2337/dc09-0479.
8. Ferenczy A, Franco E. Persistent human papillomavirus infection and cervical neoplasia. Lancet Oncol. 2002;3(1):11–6. doi:10.1016/s1470-2045(01)00617-9.
9. Yang C, Zeisberg M, Lively JC, Nyberg P, Afdhal N, Kalluri R. Integrin alpha1beta1 and alpha2beta1 are the key regulators of hepatocarcinoma cell invasion across the fibrotic matrix microenvironment. Cancer Res. 2003; 63(23):8312-7.
10. Renehan AG, Zwahlen M, Minder C, O'Dwyer ST, Shalet SM, Egger M. Insulin-like growth factor (IGF)-I, IGF binding protein-3, and cancer risk: systematic review and meta-regression analysis. Lancet. 2004;363(9418):1346-53. doi:10.1016/S0140-6736(04)16044-3
11. Shen MR, Hsu YM, Hsu KF, Chen YF, Tang MJ, Chou CY. Insulin-like growth factor 1 is a potent stimulator of cervical cancer cell invasiveness and proliferation that is modulated by alphavbeta3 integrin signaling. Carcinogenesis. 2006;27(5):962-71. doi:10.1093/carcin/bgi336
12. Maile LA, Capps BE, Ling Y, Xi G, Clemmons DR. Hyperglycemia Alters the Responsiveness of Smooth Muscle Cells to Insulin-Like Growth Factor-I. Endocrinology. 2007;148(5):2435-43. doi:10.1210/en.2006-1440
13. Radhakrishnan Y, Busby WH Jr, Shen X, Maile LA, Clemmons DR. Insulin-like growth factor-I-stimulated insulin receptor substrate-1 negatively regulates Src homology 2 domain-containing protein-tyrosine phosphatase substrate-1 function in vascular smooth muscle cells. J Biol Chem. 2010;285(21):15682-95. doi:10.1074/jbc.M109.092270
14. Khandwala HM, McCutcheon IE, Flyvbjerg A, Friend KE. The effects of insulin-like growth factors on tumorigenesis and neoplastic growth. Endocr Rev. 2000;21(3):215-44. doi:10.1210/edrv.21.3.0399
15. Webster NJ, Resnik JL, Reichart DB, Strauss B, Haas M, Seely BL. Repression of the insulin receptor promoter by the tumor suppressor gene product p53: a possible mechanism for receptor over expression in breast cancer. Cancer Res. 1996;56(12):2781-8.

16. Li G, Barrett EJ, Wang H, Chai W, Liu Z. Insulin at physiological concentrations selectively activates insulin but not insulin like growth factor (I) or insulin/IGF-1 hybrid receptors in endothelial cells. Endocrinology. 2005;146(11):4690-6. doi:10.1210/en.2005-0505
17. Sciacca L, Le Moli R, Vigneri R. Insulin analogs and cancer. Front Endocrinol (Lausanne). 2012;3:21. doi:10.3389/fendo.2012.00021
18. Fernández P. Determinación del tamaño muestral. In: Metodología de la Investigación. Fisterra, Elsevier España. 1996. p. 138-14.
19. NORMA Oficial Mexicana NOM-062-ZOO-1999, Especificaciones técnicas para la producción, cuidado y uso de los animales de laboratorio. Diario Oficial de la Federación, 2001. https://www.gob.mx/cms/uploads/attachment/file/203498/NOM-062-ZOO-1999_220801.pdf.
20. Committee for the Update of the Guide for the Care and Use of Laboratory Animals guide for the Care and Use of Laboratory Animals, The National Academies Press, 2010. http://oacu.od.nih.gov/regs/USGovtPrncpl.htm; accessed May 10, 2010.
21. NORMA Oficial Mexicana NOM-087-ECOL-SSA1-2002, Protección ambiental - Salud ambiental - Residuos peligrosos biológico-infecciosos - Clasificación y especificaciones de manejo. http://www.salud.gob.mx/unidades/cdi/nom/087ecolssa.html.
22. Medina LA, Herrera-Panilla BI, Castro-Morales MA, García-López P, Jurado R, Pérez-Cárdenas E*et al*. Use of an orthovoltage X-ray treatment unit as a radiation research system in a small-animal cancer model. J Exp Clin Cancer Res. 2008;27(1):57. doi:10.1186/1756-9966-27-57
23. Smolev JK, Coffey SD, Scott WW. Experimental models for the study of prostatic adenocarcinoma. J 1977;118(1Pt2):217-20. doi:10.1016/s0022-5347(17)57949-5
24. Alisson-Silva F, Freire-de-Lima L, Donadio JL, Lucena MC, Penha L, Sá-Diniz JN *et al*. Increase of O-Glycosylated Oncofetal Fibronectin in High Glucose-Induced Epithelial-Mesenchymal Transition of Cultured Human Epithelial Cells. PLoS One. 2013;8(4):e60471. doi:10.1371/journal.pone.0060471
25. Piątkiewicz P, Czech A. Glucose metabolism disorders and the risk of cancer. Arch Immunol Ther Exp (Warsz). 2011;59(3):215–230. doi:10.1007/s00005-011-0119-0
26. Szablewski L. Expression of glucose transporters in cancer. Biochim Biophys Acta. 2012;1835(2):164-9. doi:10.1016/j.bbcan.2012.12.004
27. Lopez R, Arumugam A, Joseph R, Monga K, Boopalan T, Agullo P *et al*. Hyperglycemia enhances the proliferation of non-tumorigenic and malignant mammary epithelial cells through increased leptin/IGF1R signaling and activation of AKT/mTOR. PLoS One. 2013;8(11):e79708. doi:10.1371/journal.pone.0079708
28. Devaraj S, Venugopal SK, Singh U, Jialal I. Hyperglycemia induces monocytic release of interleukin-6 via induction of protein kinase c-{alpha} and -{beta}. 2005;54(1):85–91. doi:10.2337/diabetes.54.1.85
29. Gonzalez Y, Herrera MT, Soldevila G, Garcia-Garcia L, Fabián G, Pérez-Armendariz EM *et al*. High glucose concentrations induce TNF-α production through the down-regulation of CD33 in primary human monocytes. BMC Immunol. 2012;13:19. doi:10.1186/1471-2172-13-19

30. Liu CJ, Chang WJ, Chen CY, Sun FJ, Cheng HW, Chen TY *et al.* Dynamic cellular and molecular modulations of diabetes mediated head and neck carcinogenesis. 2015;6(30):29268–84. doi:10.18632/oncotarget.4922
31. Masur K, Vetter C, Hinz A, Tomas N, Henrich H, Niggemann B *et al.* Diabetogenic glucose and insulin concentration modulate transcriptom and protein levels involved in tumor cell migration, adhesion and proliferation. Br J Cancer. 2011;104(2):345–52. doi:10.1038/sj.bjc.6606050
32. Quinn KA, Treston AM, Unsworth EJ, Miller MJ, Vos M, Grimley C, Battey J *et al.* Insulin-like growth factor expression in human cancer cell lines. J Biol Chem. 1996;271(19):11477–83. doi:10.1074/jbc.271.19.11477
33. Surmacz E, Guvakova MA, Nolan MK, Nicosia RF, Sciacca L: Type I insulin-like growth factor receptor function in breast cancer. Breast Cancer Res Treat. 1998;47(3):255–67. doi:10.1023/a:1005907101686
34. Nakanishi Y, Mulshine JL, Kasprzyk PG, Natale RB, Maneckjee R, Avis I *et al.* Insulin-like growth factor-I can mediate autocrine proliferation of human small cell lung cancer cell lines in vitro. J Clin Invest. 1988;82(1):354–9. doi:10.1172/JCI113594
35. Wong M, Holdaway IM: Insulin binding by normal and neoplastic colon tissue. Int J Cancer. 1985;35(3):335–41. doi:10.1002/ijc.2910350309
36. Pavelić K, Popović M. Insulin and glucagon secretion by renal adenocarcinoma. Cancer. 1981;48(1):98–100. doi:10.1002/1097-0142(19810701)48:1<98::aid-cncr2820480119>3.0.co;2-a
37. Aljada A, Saleh AM, Al Suwaidan S. Modulation of insulin/IGFs pathways by sirtuin-7 inhibition in drug-induced chemoreistance. Diagn Pathol. 2014;9:94. doi:10.1186/1746-1596-9-94
38. Zhang H, Pelzer AM, Kiang DT, Yee D. Down-regulation of type 1 insuline-like growth factor receptor increases sensitivity of breast cancer cells to insulin. Cancer Res. 2007;67(1):391–7. doi:10.1158/0008-5472.CAN-06-1712
39. Novosyadlyy R, Lann DE, Vijayakumar A, Rowzee A, Lazzarino DA, Fierz Y *et al.* Insulin-mediated acceleration of breast cancer development and progression in a nonobese model of type 2 diabetes. Cancer Res. 2010;70(2):741–51. doi:10.1158/0008-5472.CAN-09-2141
40. Clemmons DR, Maile LA, Ling Y, Yarber J, Busby WH. Role of the integrin alphaVbeta3 in mediating increased smooth muscle cell responsiveness to IGF-I in response to hyperglycemic stress. Growth Horm IGF Res. 2007;17(4):265–70. doi:10.1016/j.ghir.2007.01.004.
41. Villarreal-Garza C, Shaw-Dulin R, Lara-Medina F, Bacon L, Rivera D, Urzua L, *et al*. Impact of diabetes and hyperglycemia on survival in advanced breast cancer patients. Exp Diabetes Res. 2012;2012:732027. doi:10.1155/2012/732027
42. Gao M, Ma Y, Liu D. High-fat diet-induced adiposity, adipose inflammation, hepatic steatosis and hyperinsulinemia in outbred CD-1 mice. PLoS One. 2015;10(3):e0119784.doi:10.1371/journal.pone.0119784

Figures

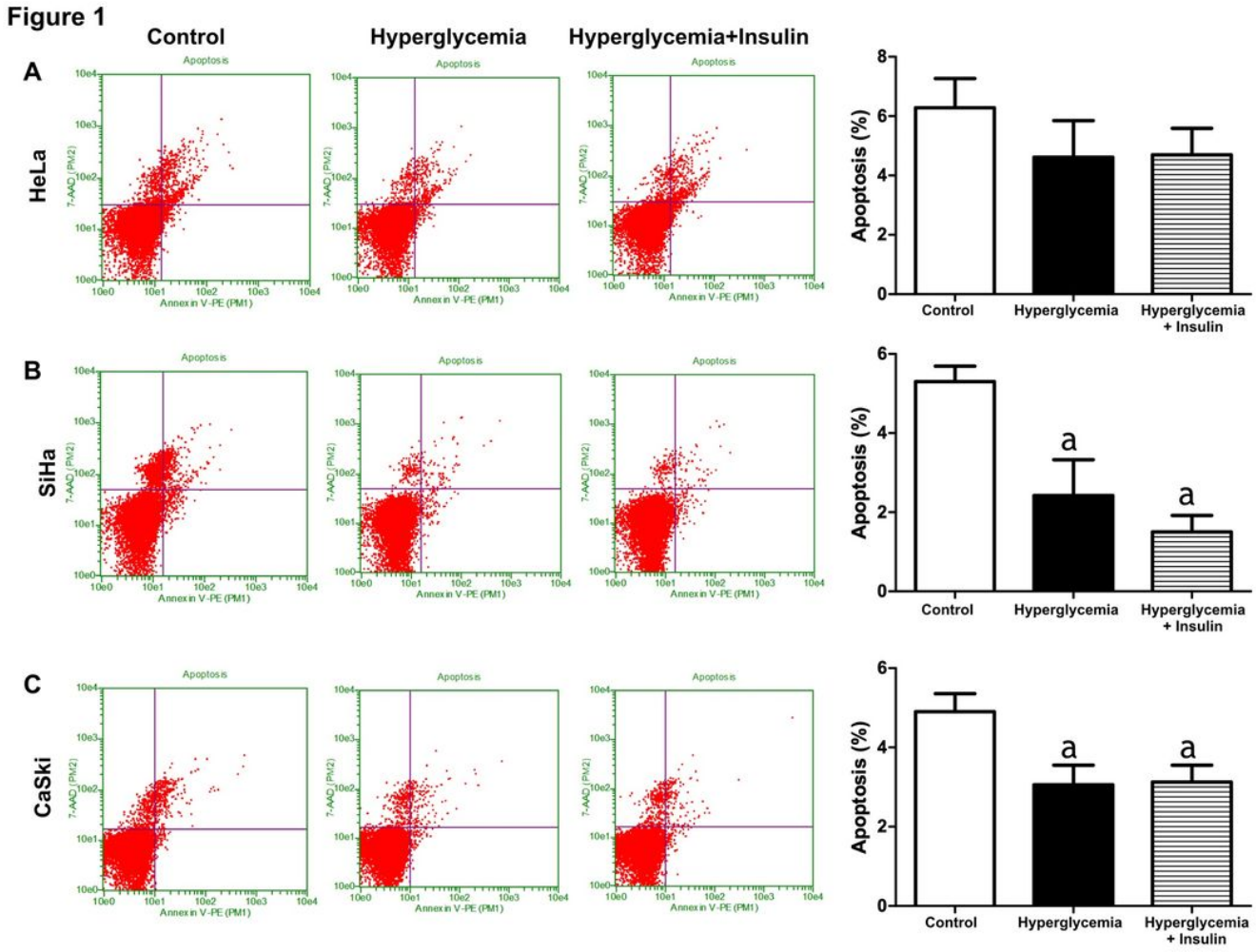
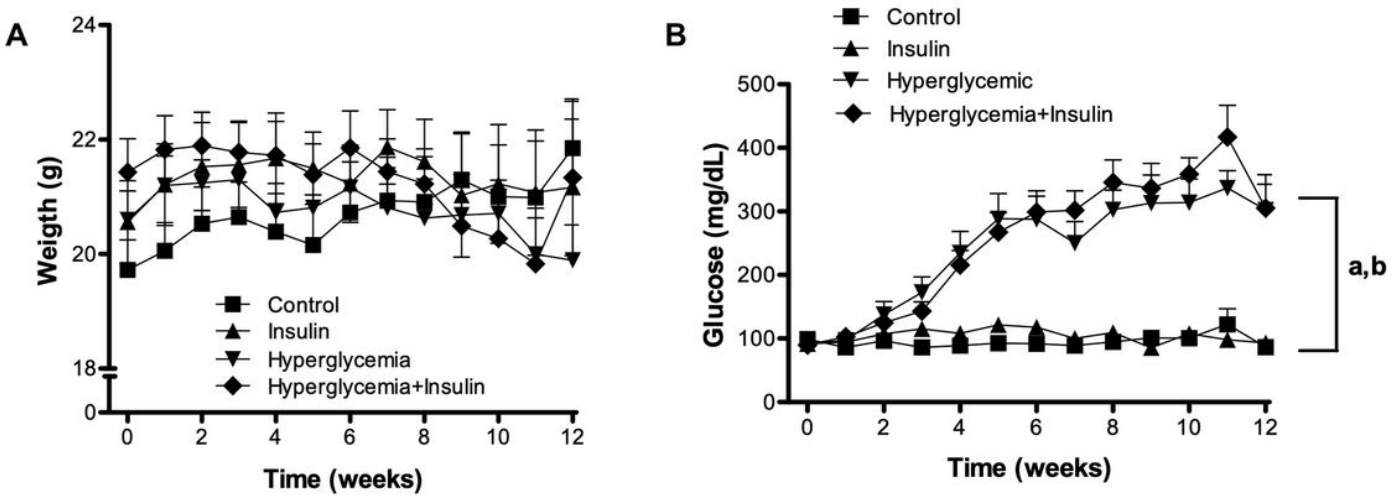


Figure 1

Total apoptosis (%). A) HeLa cell line, B) SiHa cell line, C) CaSki cell line. The left side figure shows representative dot plots of anexin and 7-AAD flow cytometry in control, hyperglycemia and hyperglycemia + insulin (H+I) groups, right side show analysis of total apoptosis (%). A) HeLa cell line B) SiHa cell line, C) CaSki cell line. Each bar represents the mean ± SEM of three independent experiments. a $P \leq 0.05$ vs control group.

Figure 2



C

	Glucose	Cholesterol	Triglycerides	Creatinine	BUN
Control	118 ± 9.3	47.3 ± 1.8	59.5 ± 6.2	0.73 ± 0.06	18.3 ± 1.8
Control+insulin	117 ± 8.7	56.6 ± 2.2	58.8 ± 8.5	0.99 ± 0.09	23.8 ± 1.1
Hyperglycemia	312 ± 25.4 [a]	70.5 ± 4.6 [a]	93.9 ± 10.5 [a]	1.26 ± 0.15 [a]	25.3 ± 1.7 [a]
Hyperglycemia+insulin	337 ± 37.7 [a]	76.1 ± 9.9 [a]	69.6 ± 8.8	1.22 ± 0.13 [a]	26.4 ± 2.2 [a]

Figure 2

Blood glucose levels, body weight and biochemical parameters. A) Temporal course of blood glucose measured once a week in all groups (control, insulin, Hyperglycemia and Hyperglycemia+Insulin). B) Body weight measured once week of mice treated with vehicle (control group), insulin, STZ (hyperglycemic group) and STZ+insulin (Hyperglycemia+Insulin). C) Biochemical parameters of all groups at the end of the experiment. Each point represents the mean ± SEM of 9 to 13 mice. a $P \leq 0.05$ vs control group; b $P \leq 0.05$ vs Insulin group.

Figure 3

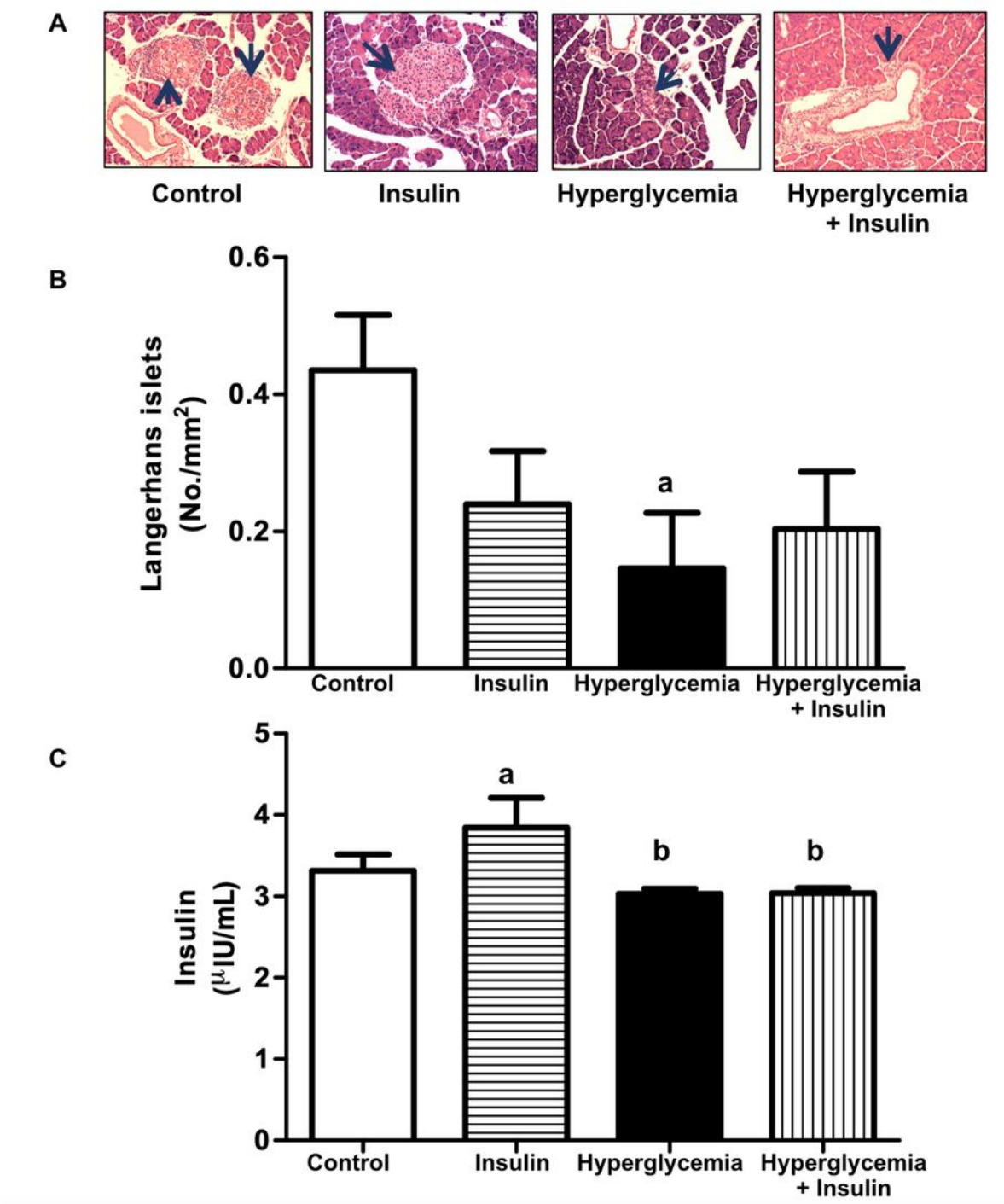
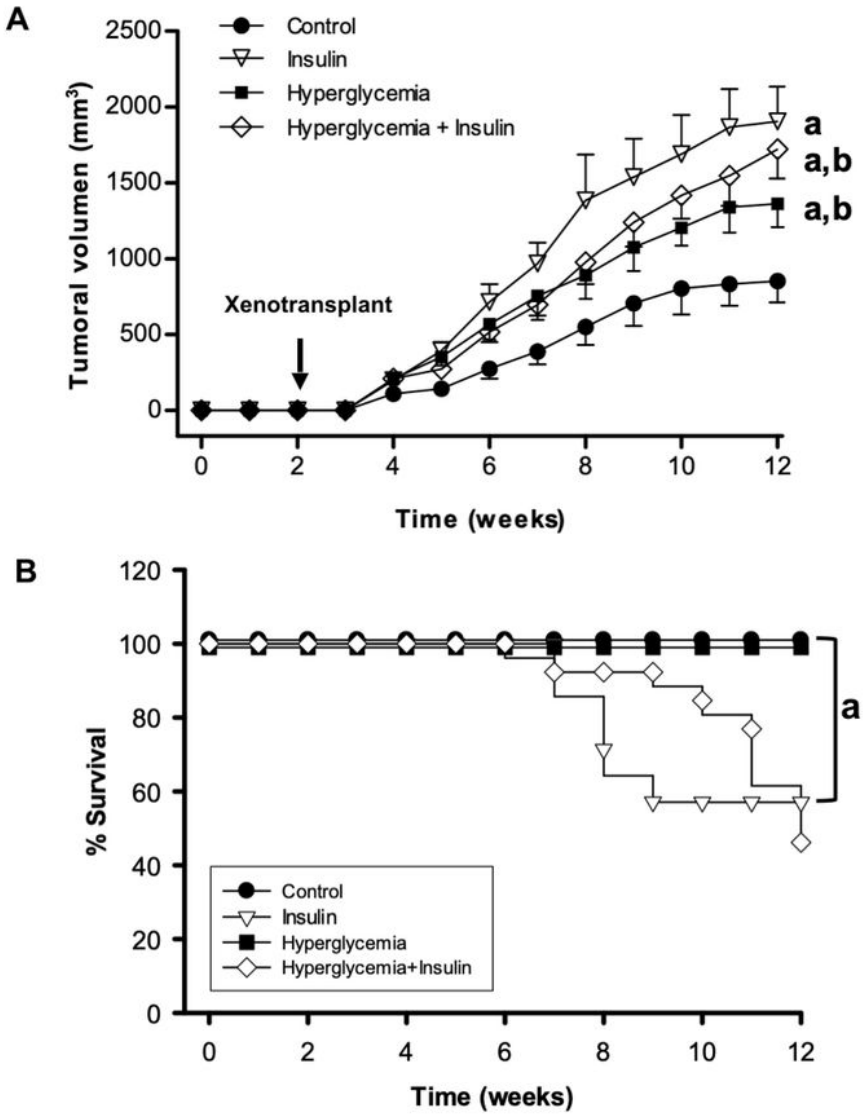


Figure 3

A) a representative micrography of Langerhans islets stained with H&E, arrows mark an islet in all experimental groups. B) Number of Langerhans islets per mm2 in all experimental groups. C) Serum insulin levels in all experimental groups. a $P \leq 0.05$ vs control group, b $P \leq 0.05$ vs Insulin group. Results are expressed as mean ± SEM of 9 to 13 mice.

Figure 4



C

Groups	n	Tumor volume (mm³) Initial	Final	Cell doubling time (days)
Control	9	165 ± 10.9	853 ± 140.6	22.2 ± 2.6
Control+insulin	7	178 ± 33.3	1769 ± 236.5 [a]	13.1 ± 1.7 [a]
Hyperglycemic	10	180 ± 25.4	1269 ± 166.5 [a]	17.1 ± 1.0 [a]
Hyperglycemia+insulin	13	171 ± 24.3	1404 ± 191.6 [a]	15.0 ± 1.3 [a]
P		NS	≤ 0.05	≤ 0.05

Figure 4

Tumoral volume. A) Temporary course of tumors from transplanted HeLa cells in nude mice under hyperglycemic and/or hyperinsulinemia conditions. B) Percentage of survival in the fourth study groups. C) Growth of xenotransplants of HeLa cells in hyperglycemic conditions, the nude mice were treated with or without insulin for 12 weeks. a $P \leq 0.05$ vs control group, b $P \leq 0.05$ vs Insulin group. Results are expressed as mean ± SEM of 9 to 13 mice.

Supplementary Files

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- NC3RsARRIVEGuidelinesChecklist.docx