Direct Evidence for Intracellular Homeostasis in Mammalian Cells: Insulin-independent Glucose Metabolisms

Abstract

In our previous study, carbonates, $NaHCO_3$ and Na_2CO_3, influence glucose metabolism in vitro, using Py-3Y1-S2 rat fibroblast cells, and these compounds accelerate significantly glucose consumption. In the present study, the effects of the carbonates on glucose metabolism were examined to determine whether these effects are universal among different cell lines, VERO green monkey kidney cells, TE-13 human esophageal cancer cells, and HepG2 human cells. Glucose was completely converted to lactate, which disappeared gradually from the culture medium. However, the disappearance of lactate from the medium was independent of carbonates. The present study clarified that $NaHCO_3$ and Na_2CO_3 directly regulate glucose metabolism among different cell lines via an insulin-independent pathway, that is, intracellular homeostasis.

Keywords: Intracellular, Homeostasis, Insulin, Glucose, Metabolism, Diabetes, Anti-diabetic, Carbonates, Lactate, Concanavalin A, Vanadium, Mitochondria.

1. Introduction

Blood sugar levels are regulated *in vivo* by insulin and glucagon, which are produced from ß and α cells, respectively, of the pancreatic islets, via homeostatic mechanisms, which maintain *in vivo* vertebrate life. This is a delicate mechanism, which has evolved in vertebrates along with long-term evolution of other biological differentiated functions. The present study aims to clarify the existence of a regulatory mechanism for cellular glucose metabolism in the absence of insulin before establishment of the endocrine system, in which insulin plays an important role in cells.

Regarding its mode of action as a peptide hormone, insulin binds to its receptor on the plasma membrane, leading to intramolecular phosphorylation within the activated receptor as a tyrosine kinase. The signal transduction proceeds through phosphatidylinositol-3-kinase, protein kinase B, and glucose transporter-4 (GLUT-4), with GLUT-4 plus K^+ accelerating glucose uptake into cells [1]. Vanadium compounds were reported to exhibit insulin-like activity not only *in vitro* [2,3], but also *in vivo* [4-11], and several vanadium compounds have been investigated for their insulin-like activity [12-16]. The insulin-like effects of vanadates are based on inhibition of protein-tyrosine phosphatase [17]. However, to our knowledge, suitable

vanadium compounds have not yet been developed as anti-DM drugs because of the serious cytotoxic effects of high vanadium concentrations. It has been suggested that Mt. Fuji subsoil water filtered through basalt can exhibit insulin-like activity, because the water contains vanadium pentoxide (V_2O_5) *in vivo* [18]. Recently, we confirmed that Mt. Fuji subsoil water accelerates glucose consumption *in vitro* using established Py-3Y1-S2 rat fibroblast cells [19] and human primary fibroblasts [20]. Vanadium pentoxide is soluble in the alkaline condition, but its water solubility is quite low (0.7–0.8 g/L). Indeed, the pH value of commercial Mt. Fuji subsoil water (Healthy Vana Water) containing 130 μg/L vanadium was 8.3 [19]. If vanadium-containing water can be prepared by mixing a small amount of Mt. Fuji basalt powder with normal water, the vanadium-containing water could be conveniently used instead of Mt. Fuji subsoil water.

In our previous study [21], established Py-3Y1-S2 rat fibroblast cells were used to evaluate whether $NaHCO_3$ or Na_2CO_3 influences glucose metabolism *in vitro*, because factors that contribute to metabolic pathways are much simpler to evaluate in cultured cells than in whole animal bodies. The effects of the carbonates on glucose consumption decreased at high concentrations, >5 mg/ml for Na_2CO_3 and >7 mg/ml for

$NaHCO_3$, because of the increased pH of the culture medium. The effects of the carbonates on glucose consumption were additive with those of vanadium and concanavalin A. Streptozotocin, alloxan, and nicotinamide, which induce diabetes in animals, reduced glucose consumption by Py-3Y1-S2 cells, and the inhibitory effects of these reagents were abolished by both Na_2CO_3 and $NaHCO_3$. Finally, the carbonates increased lactate production from glucose in the cells, followed by acceleration of lactate secretion into the culture medium.

2. Materials and Methods

The source of chemicals was described in the previous papers [19-21].

The method of cell culture, glucose assay, lactate assay, and protein assay were precisely described in the previous papers [19-21]. Cell lines: VERO green monkey kidney cells [22], TE-13 human esophageal cancer cells [23], and HepG2 human cells [24] were used.

Statistical calculations by the *t*-test were performed using Microsoft Excel (version 2010). Values of $p < 0.05$ and $p < 0.01$ were considered significant and highly significant, respectively.

3. Results

3.1 Different cell lines. To confirm that carbonates, i.e., $NaHCO_3$ and Na_2CO_3, contribute to glucose consumption in cellular metabolisms as a general rule,

several different established cell lines were examined. TE-13 cells were derived from human esophageal cancer [23]. These cells were used in our previous study with Mt. Fuji subsoil water, which contains vanadium pentoxide (V_2O), and vanadium ions accelerated glucose consumption [19,20]. In addition, VERO and HepG2 were examined. When the cells were cultured in a medium to which 1.0 mg/ml $NaHCO_3$ or Na_2CO_3 had ben added, glucose consumption was significantly accelerated (Fig. 1A). The effects of carbonates on glucose consumption varied slightly among different cell lines. After culturing for a sufficient time period, the glucose in the medium in the 24-well culture plates was almost completely consumed, whereas lactate production reached a plateau. There was no significant difference between lactate production by the control cells and that by the carbonate-treated cells, and among different cell lines (Fig. 1B). The plateau concentration of lactate was 10–12 mmol/L. This value is twice the initial glucose concentration (5.6 mM). This suggests that glucose in the medium was completely converted to lactate in these cell lines.

3.6 Lactate metabolism. When Py-3Y1-S2 cells were continuously cultured, the lactate concentration decreased gradually with incubation time (Fig. 2). After culturing for 2 days, about

50% reduction was observed, and after 3 days more than 60% of the lactate had disappeared. No significant effect of carbonates on lactate reduction was observed (Fig. 2). Eventually, the carbonates apparently accelerated only glucose consumption. Similar lactate reduction occurred in the other cell lines, i.e., TE-13, VERO, and HepG2, although the reduction rates differed. The fact that the secreted lactate from cells was further metabolized by cells seems to have a certain physiological functional significance. The rate difference between glucose consumption and lactate reduction may contribute to maintenance of a cellular steady state, i.e., intracellular homeostasis. Lactate was largely considered a dead waste product of glycolysis due to hypoxia, the primary cause of O_2 debt following exercise, a major cause of muscle fatigue. However, its physiological significance has been reevaluated [24].

3.7 Non-insulin effect. To determine whether insulin receptors are involved in glucose consumption by carbonates in cells, Py-3Y1-S2 cells were cultured in the presence of insulin. No significant acceleration of glucose consumption was observed in the range 0.1–50 μg/ml insulin (Fig. 3).

4. Discussion

Plants and some bacteria are

autotrophs and are able to grow by using photosynthetic energy, CO_2, and H_2O. Other autotrophs are the chemolithotrophs, which use an inorganic substrates such as hydrogen or thiosulfate as a reductant and carbon dioxide as a carbon source. However, animals and many bacteria, except for the above-mentioned autotrophic bacteria, require organic carbon for growth via catabolism and anabolism, which involve biochemical reactions using chemical energy. These biochemical reactions occur in living cells as well as in cell growth. In general, to produce chemical energy, glucose or hydrolyzed carbohydrates are used as nutrients. Differentiation of vertebrates has led to blood sugar levels in whole bodies being maintained *in vivo* by the actions of insulin and glucagon. However, not only single-cell organisms such as bacteria and protozoa, but also multicellular organisms such as invertebrates, have certain primitive glucose regulatory mechanisms which enable them to survive without an endocrine system. It would not be surprising if these less-developed organisms have characteristic carbohydrate metabolisms which differ from the endocrine system established in vertebrates. In addition, these primitive glucose regulatory mechanisms could still be preserved in vertebrate cells under dedifferentiated conditions.

Cell cultures provide a useful

tool for investigating cellular metabolisms *in vitro* because such a system is much simpler than a whole body, which consists of various different cells and metabolic pathways *in vivo*. In 1955–1959, Eagle and his coworkers developed a method for culturing isolated cells *in vitro* [25]. His developed medium for *in vitro* cell culture, i.e., Eagle's minimum essential medium (MEM), consists of amino acids, glucose, vitamins, and salts. In certain cases, 5%–10% bovine serum is added. Some amino acids such as alanine, asparagine, aspartic acids, glycine, hydroxyproline, proline, and serine have been removed from the medium because these amino acids are biosynthesized in cells. It is possible to remove serum from certain cell cultures, and glutamine and tyrosine have been deleted from the medium for rat hepatoma cells, Ry121B [26]. Sato and coworkers added hormones to a chemically defined culture medium instead of serum [27,28]. (To our knowledge, however, a culture medium which does not contain glucose has not yet been developed.) Glucose is essential for organisms, except autotrophs, for energy production and to provide a carbon source. Various culture media such as BME [29], MEM [30], Fisher [31], F12 [32], RPMI [33], DM-160 [34], and DME [35] contain 900–2,000 mg/L glucose. My former supervisor, Emeritus Professor Yasumura, who established famous cell lines such as VERO [22], Y1 [36] and GH [37] tried to

establish cells which can grow in a glucose-free chemically defined medium, by using green monkey kidney cell VERO. However, he was unable to complete this work during his research life at the Dokkyo Medical University.

Glucose metabolism has been completely clarified based on biochemical reactions, not only in prokaryotes but also in eukaryotes, and glucose metabolisms are almost the same among various organisms. However, some biochemical reactions occur at different places in prokaryotes than in eukaryotes. One major difference between the cellular structures is the presence of mitochondria in eukaryotes, and this organelle contributes to the respiratory function, which metabolizes carbohydrates, i.e., glucose. In general, one molecule is converted to two pyruvate molecules, and finally converted to CO_2 and H_2O via the tricarboxylic acid (TCA) cycle in the presence of oxygen. In contrast, in the absence of oxygen, pyruvate produced from glucose is converted to lactate via the Embden–Meyerhof pathway or the alternative Entner–Doudoroff pathway. Eventually, one glucose molecule is converted to two lactate molecules in the absence of oxygen. In the present study, the glucose contained in the culture medium was almost all converted to lactate, although the cells were cultured in the presence of oxygen (Fig. 1). This indicates that the TCA cycle is not

involved in glucose metabolism in the cultured cells, i.e., glucose was metabolized via an aerobic pathway to lactate in the cells.

In prokaryotes, the complete genome of *Mycoplasma plumonis* consists of 782 protein genes and 963,879 nucleotides [38], and that of *Ureaplasma urealyticum* consists of 646 protein genes and 874,478 nucleotides [39]. In eukaryotes, the *Homo sapiens* (human) genome consists of 20,109 protein genes and 2,851,330 mb nucleotides [40,41]. These facts indicate that biological evolution diverged along with increases in the number of protein genes and nucleotide numbers in chromosomal DNA. Glucose metabolism maintains life not only in prokaryotes but also in eukaryotes, which have mitochondria. The *Reclinomonas americana* (Protist) (~70 kb), consisting of 97 genes, is thought to be an ancestral mitochondrial DNA, whereas vertebrate mitochondrial DNA (~16 kb), consisting of 13 respiratory genes, seems to be constructed with only essential genes for respiration reactions. These decreases in protein and total nucleotide numbers along with mitochondrial evolution are the reverse of the phenomena observed in chromosomal DNA. It has been suggested that mitochondria developed from the protobacterium *Rickettsia* or its relatives, on the basis of gene similarities between these two cellular organelle DNAs [42,43].

We showed that normalization of the nucleotide contents of a complete genome indicates the characteristics of an organism [44]. For example, this procedure was used to classify prokaryotes into two groups, namely *Escherichia coli* and *Staphylococcus aureus* types [45], and for construction of phylogenetic trees [46]. The use of normalized nucleotide values enables certain nucleotide contents to be expressed by a linear regression line [47]; for example, the cytosine content can be expressed by $C = aG + b$, where C and G are nucleotide contents and a and b are constants, based on Chargaff's second parity rule [48], as shown in Fig. 4. The regression lines obtained from complete chromosomal, plant mitochondrial, and chloroplast DNA overlap, whereas those obtained from complete animal mitochondrial DNA deviate from these regression lines. Although it was reported that mitochondrial DNA deviated from Chargaff's second parity rule [48], two regression lines can be obtained, dividing animal mitochondria into two groups, namely groups with high and low C/G contents [47,49]. As *Monosiga brevicollis* mitochondria have the lowest C/G contents among all cellular organelles, as shown in Fig. 4, we concluded that *Monosiga brevicollis* mitochondria may be the most primitive extant ancestor of the species examined [50]. In addition, the fact that all the regression lines

crossed at a single point indicates that all organisms might diverge from a single origin of life [48,50], as speculated in Darwin's theory. Our previous study indicated that more highly evolved organisms have greater normalized cytosine contents in their complete genomes, and the highest cytosine content was observed in primate and avian complete mitochondrial genomes [48,50]. This is consistent with results based on complete genome analysis, which were reported by another group [51]. In contrast, the normalized cytosine contents of plant mitochondrial genomes which obey Chargaff's second parity rule [48] showed lower evolutionary divergence than in the case of vertebrate mitochondrial genomes [47,50]. Vertebrate mitochondrial evolution therefore seems to be linked with expansion of animal active behaviors, which consume a lot of energy. The numbers of mitochondria in the liver, kidney, muscle, and brain are larger than those in other organs. Cells cultured *in vitro* might not need a large number of mitochondria, which produce energy, because their mobility is limited. In the presence of oxygen, the TCA cycle produces CO_2 and H_2O as the final products of glucose metabolism. This means that a carbon source is lost from the system, whereas lactate, which is produced from glucose in the absence of oxygen, can be reused later in the system.

In the present study, many cell lines produced lactate as the final product from glucose (Fig. 1). These results indicate that the Embden–Meyerhof and Entner–Doudoroff pathways are active in cultured cells, even if oxygen is present. Lactate metabolism by cells was slower than glucose consumption (Fig. 2). The lactate production pathway therefore seems to assist a rapid decrease in the blood glucose level *in vivo*. In addition, the rapid secretion of lactate into the culture medium is necessary for cells to maintain a neutral pH inside the cells, and the metabolism of lactate secreted from cells may contribute clinically to recovery from lactate acidosis. The results of the present study indicate that mammalian cells basically have to metabolize extracellular lactate.

It is well known that tissue cultures lose their differentiated cellular functions *in vitro*. It is therefore impossible to establish cell lines which reserve full organ specific functions, and only certain differentiated functions are randomly maintained. To our knowledge, there is no cell line in which gluconeogenesis takes place *in vitro*. In addition, the control of blood sugar levels is based on homeostasis by the endocrine system, which is established in highly evolved organisms such as vertebrates. This endocrine system is also a differentiated function. In the present study, glucose metabolism of Py-3Y1-S2

cells was independent of insulin (Fig. 3), although insulin receptors are present on the plasma membranes of various established cell lines [52]. However, addition of carbonates, namely $NaHCO_3$ and Na_2CO_3, to the culture medium accelerated glucose consumption [21]. It is therefore clear that there is an insulin-independent glucose metabolic pathway in Py-3Y1-S2 cells. The addition of $NaHCO_3$ to the basic culture medium maintains a neutral pH in a 5% CO_2 incubator, rather than cell nutrient or glucose metabolism regulation.

Con A [21,52] and vanadium compounds [7-22] showed insulin-like activity not only *in vivo* but also *in vitro*. Our present study and previous [18-20] studies confirmed these results for Py-3Y1-S2 cells. Con A is a lectin and protein, and vanadium is a metal and its salts are metal compounds. Carbonates such as $NaHCO_3$ and Na_2CO_3 are inorganic compounds. Their molecular structures clearly differ not only from that of insulin but also from those of Con A or vanadium. Carbonates seem not to bind to insulin receptors on the plasma membrane to induce signal transductions, followed by activation of a glucose transporter (GLUT 4). In addition, the dissociation constants (K_d) of insulin with its receptors on cultured rat hepatoma cells (Ry121B) were ~4 $x10^{-9}$ M and ~3 $x10^{-8}$ M, at the high and low insulin-binding sites, respectively [53]. These values are much

lower than ~1 mg/ml (17 mM) of $NaHCO_3$ [21]. The acceleration of glucose consumption by these small molecules therefore takes place via an insulin receptor-independent pathway.

Addition of nicotinamide, which is normally present in the basic culture medium, reduced glucose consumption, but this inhibitory effect was abolished by carbonates [21]. Nicotinamide, alloxan, and STZ induce diabetes [54-56]; this is consistent with the present results. Insulin acts on target tissues to reduce blood glucose levels. However, the present study indicates that insulin-independent glucose metabolisms occurs in cells. It is necessary to consider this newly discovered pathway to achieve a more precise understanding of glucose metabolisms, not only *in vitro* but also *in vivo*.

5. Conclusions

The present study indicates that $NaHCO_3$ and Na_2CO_3 directly regulate glucose metabolism in Py-3Y1-S2 cells, VERO green monkey kidney cells, TE-13 human esophageal cancer cells, and HepG2 human cells via insulin-independent cellular glucose metabolisms based on intracellular homeostasis.

Acknowledgments

The author thanks President Hiroyuki Okada of Shinko Sangyo Co. Ltd. (Takasaki, Gunma, Japan) for his financial

support, Dr. Kazumi Akimoto of Dkkyo Medical University for his kind supply of several cell lines, and Mr. Fumio Takei of Fuji Yogan Institute (Yamanashi, Japan) for his kind donation of Mt. Fuji basal powder. The author also thanks Alison Sherwin, PhD, from Edanz Group (www.edanzediting.com/ac) for editing a draft of this manuscript.

Funding

All costs for the present research were covered by Sinko Sangyo Co. Ltd. (Takasaki, Gunma, Japan).

Conflict of Interest

The present work has been used for the application of patents.

References

1. De Meyts P. In: Feingold KR, Anawalt B, Boyce A, Chrousos G, Dungan K, Grossman A, Hershman JM, Kaltsas G, Koch C, Kopp P, Korbonits M, McLachlan R, Morley JE, New M, Perreault L, Purnell J, Rebar R, Singer F, Trence DL, Vinik A, Wilson DP, editors. Endotext [Internet]. South Dartmouth (MA): MDText.com, Inc.; 2000-. The Insulin Receptor and Its Signal Transduction Network. 2016 Apr 27.
2. Chechter Y, Karlish SJ. Insulin-like stimulation of glucose oxidation in

rat adipocytes by vanadyl (IV) ions. Nature 1980; 284: 556-8.

3. Duckworth WC, Solomon SS, Liepnieks J, Hamel FG, Hand S, Peavy DE.Insulin-like effects of vanadate in isolated rat adipocytes. Endocrinol 1988; 122: 2285-9.

4. Meyerovitch J, Farfel Z, Sack J, Shechter Y. Oral administration of vanadate normalizes blood glucose levels in streptozotocin-treated rats. Characterization and mode of action. J Biol Chem, 1987; 262: 6658-62.

5. Heyliger CE, Tahiliani AG, McNeill JH. Effect of vanadate on elevated blood glucose and depressed cardiac performance of diabetic rats. Science 1985; 227: 1474-7.

6. Sakurai H, Tsuchiya K, Nukatsuka M, Sofue M, Kawada J. Insulin-like effect of vanadyl ion on streptozotocin-induced diabetic rats. J Endocrinol 1990; 126: 451-9.

7. Thompson KH, Leichter J, McNeill JH. Studies of vanadyl sulfate as a glucose-lowering agent in STZ-diabetic rats. Biochem Biophy Res Comm, 1993; 197: 1549-55.

8. Xie M, Gao L, Li L, Liu W, Yan S. A new orally active antidiabetic vanadyl complex--bis(alpha-furancarboxyla

to)oxovanadium(IV). J Inorg Biochem, 2005; 99: 546-51.

9. Pillai SI, Subramanian SP, Kandaswamy M. A novel insulin mimetic vanadium-flavonol complex: synthesis, characterization and in vivo evaluation in STZ-induced rats. Euro J Med Chem 2013; 63: 109-17.

10. Jiang P, Dong Z, Ma B, *et al.* Effect of Vanadyl Rosiglitazone, a New Insulin-Mimetic Vanadium Complexes, on Glucose Homeostasis of Diabetic Mice. Appl Biochem Biotechnol 2016; May 30. [Epub ahead of print]

11. Liu Y, Xu J, Guo Y, Xue Y, Wang J, Xue C. Ameliorative effect of vanadyl(IV)-ascorbate complex on high-fat high-sucrose diet-induced hyperglycemia, insulin resistance, and oxidative stress in mice. J Trace Elem Med Biol 2015; 32: 155-61.

12. Poucheret P, Verma S, Grynpas MD, McNeill JH. Vanadium and diabetes. Mol Cell Biochem 1998; 188: 73-80.

13. Shafrir E, Spielman S, Nachliel I, Khamaisi M, Bar-On H, Ziv E. Treatment of diabetes with vanadium salts: general overview and amelioration of nutritionally induced diabetes in the Psammomys

obesus gerbil. Diabetes Metab Res Rev 2001; *17*: 55-66.

14. Rehder D. Perspectives for vanadium in health issues. Fut Med Chem 2016; 8: 325-38.

15. Kibiti M, Afolayan AJ. The Biochemical Role of Macro and Micro-Minerals in the Management of Diabetes Mellitus and its Associated Complications: A Review. Int J Vit Nut Res 2015; 85: 88-103.

16. Gruzewska K, Michno A, PawelczykT, Bielarczyk H. Essentiality and toxicity of vanadium supplements in health and pathology. J Physiol Pharmaco 2014; 65: 603-11.

17. Huyer G, Liu S, Kelly J, *et al.* Mechanism of inhibition of protein-tyrosine phosphatases by vanadate and pervanadate. J Biol Chem 1997; 272: 843-51.

18. Kitsuta T, Attractive vanadium water in decrease in blood glucose concentration (in Japanese). In Good Blood Condition with Vanadium Water. Tokyo: An extra number of Takarajima (Vol. 1054) 2004; pp. 12-22.

19. Sorimachi, K., Ohmori, Y., Matsukawa, T., Yokoyama, K. and Ohhira, S. Insulin-like effects of Mt. Fuji subsoil water which contains vanadium on cultured cells: Insight from Japan. Curr. Trad. Med. 2017;

3: 178-189.

20. Sorimachi, K., Ohhira, S. Insulin-like effects of vanadium pentoxide (V2O5) o glucose consumption in primary human fibroblasts. J Anal Pherm Res 2017; 5(4): 00150.

21. Sorimachi K. Direct evidence for glucose consumption acceleration by carbonates in cultured cells. Int J Pharma Phytopharm Res 2019; 9(3): 1-5.

22. Yasumura Y. and Kawakita Y. Studies on SV40 in tissue culture: Preliminary step for cancer research in vitro. (In Japanese), Nihon Ryinsho 1963; 21: 1201-15.

23. Oyamada Y, Oyamada M, Fusco A, Yamasaki H. Aberrant expression, function and localization of connexins in human esophageal carcinoma cell lines with different degrees of tumorigenicity. J Cancer Res Clin Onco 1994; 120: 445-53.

24. Chen L., Teng H., Cao H. Chlorogenic acid and caffeic acid from Sonchus oleraceus Linn synergistically attenuate insulin resistance and modulate glucose uptake in HepG2 cell. Food Chem Toxicol 2019; 127: 182-187.

25. Eagle H. The growth requirements of two mammalian cell lines in tissue culture. Trans Assoc Am Physicians 1955; 68: 78-81.

26. Yasumura Y., Niwa A., Yamamoto K. Phenotipic requirement for glutamine of kidney cells and for glutamine and arginine of liver cells in culture. In Nutritional Requirements of Cultured Cells. Katsuta, H. ed. 1978, pp. 223-255.Japan Scientific Society Press Tokyo, and University Park Press, Baltimore.

27. Sato G, Hayashi I. The replacement of serum by hormones in cell culture media. Arch Biol Med Exp (Santiago). 1976 Dec;10(1-3):120-1.

28. Barnes D, Sato G. Growth of a human mammary tumour cell line in a serum-free medium. Nature 1979; 281(5730): 388-9.

29. Eagle H. Nutrition needs of mammalian cells in tissue culture. Science 1959; 122 (3168): 501-14.

30. Eagle H. Amino acid metabolism in mammalian cell cultures. Science 1959; 130(3373): 432-7.

31. Fisher GA., Sartorelli AC. Development, maintenance and assay of drug resistance. Methods Med Res. 1964; 10:247-62.

32. Ham RG. Clonal growth of mammalian cells in a chemically defined medium. Proc Nat Acad Sci USA 1965; 53: 288-93.

33. Moor GE., Gerner RE., Franklin HA. Culture of normal human leukocytes. J Amer Med Assn 1967 ;199(8):519-24.

34. Katsuta H, Takaoka T. Improved synthetic media suitable for tissue cllture of various mammalian cells. Methods Cell Biol. 1976;14:145-58.

35. Dulbeccor R., Freeman G. Plaque production by the polyoma virus. Virology. 1959; 8(3):396-7.

36. Shin SI., Yasumura Y., Sato GH. Studies on interstitial cells in tissue culture. II. Steroid biosynthesis by a clonal line of rat testicular interstitial cells. Endocrinology. 1968; 82(3):614-6.

37. Yasumura, Y. Retention of differentiated function in clonal animal cell lines, particularly hormone-secreting cultures. Am Zool 1968 ;8(2):285-305.

38. Chambaud I., *et al.* The complete genome sequence of the murine respiratory pathogen *Mycoplasma pulmonis.* Nucleic Acids Research, 2001; 29 (10): 2145–53.

39. Glass JI, *et al.* The complete sequence of the mucosal pathogen *Ureaplasma urealyticum.* Nature 2000;407(6805):757-62.

40. International Human Genome Sequencing Consortium. Initial sequencing and analysis of the human genome. Nature 2001, ;409, 860–921.

41. Venter JC., *et al.* The sequence of the human genome. Science 2001; 291(5507):1304-51.

42. Andersson, S.G. *et al*. The genome sequence of Rickettsia prowazekii and the origin of mitochondria. Nature 1998; 396: 133-140.

43. Thrash, J.C. *et al*. Phylogenomic evidence for a common ancestor of mitochondria and the SAR11 clade. Sci Rep 2011;1:13.

44. Sorimachi K, Okayasu T, Ohhira S. Normalization of Complete Genome Characteristics: Application to Evolution from Primitive Organisms to Homo sapiens. Curr Genomics 2015;16(2):99-106.

45. Sorimachi K., Okayasu T. Classification of eubacteria based on their complete genomes: where Mycoplasmataceae belong? Proc R Soc Lond (Suppl), 2004; 271: S127-S130.

46. Sorimachi K, Okayasu T. Phylogenetic tree construction based on amino acid composition and nucleotide content of complete vertebrate mitochondrial genomes. IOSR J Pharm. 20013; 3: 51-60.

47. Sorimachi K. Origine of life in the ocean: direct derivation of mitochondria from primitive organisms based on complete genomes. Cur Chem Biol 2015; 9: 23-35.

48. Rundner R, Karkas JD, Chargaff E. Separation of *B. subtilis* DNA into complementary strands. 3. Direct analysis. Proc Natl Acad Sci USA 1968; 60: 921-922.

49. Sorimachi K. The most primitive extant ancestor of organisms and discovery of definitive evolutionary equations based on complete genome structures. Natl Sci 2018; 10 (9): 338-369.

50. King N. *et al.* The genome of the choanoflagellates *Monosiga brevicollis* and the origin of metazoans. Nature 2008; 451: 783-788.

51. Sorimachi K., Niwa A., Yasumura Y. “Blocking” and “trapping” effects of concanavalin A on insulin binding in various cell lines. Dokkyo J Med Sci 1984; 11: 11-17.

52. Cuatrecasas P., Tell GPE. Insulin-like activity of concanavalin A and wheat germ agglutin-Direct interaction with insulin receptors. Proc Nat. Acad Sci USA 1973; 70 (2): 485-489.

53. Sorimachi K., Okayasu T., Yasumura Y. Increase in insulin binding affinity by chloroquine in cultured rat hepatoma cells. End Res 1987; 13 (1): 49-60.

54. Szkudelski T. The mechanism of alloxan and streptozotocin action in B cells of the rat pancreas. Physiol Res 2001; 50: 536-546.

55. Ghasemi A., Khalifi S., Jedi S. Streptototocin-nicotinamde-induced rat model of type 2 diabetes (review). Acta Physiol Hung 2014; 101 (4): 408-420.

56. Holland WL, *et al.* Lipid-induced insulin resistance mediated by the proinflammatory receptor TLR4 requires saturated fatty acid-induced ceramide biosynthesis in mice. J Clin Invest 2011; 121(5):1858-70.

Figure Legends

Figure 1. Effect of $NaHCO_3$ or Na_2CO_3 on glucose consumption (A) and lactate production (B) by different cell lines. Data represent means ± SD of 6–8 independent experiments. *$p < 0.05$; **; $p < 0.01$.

Figure 2. Gradual decrease in lactate concentration in culture medium treated with

Py-3Y1-S2 cells. A 10 µl sample of culture medium was removed after incubation for 1, 2, 3, and 4 days for lactate assays.

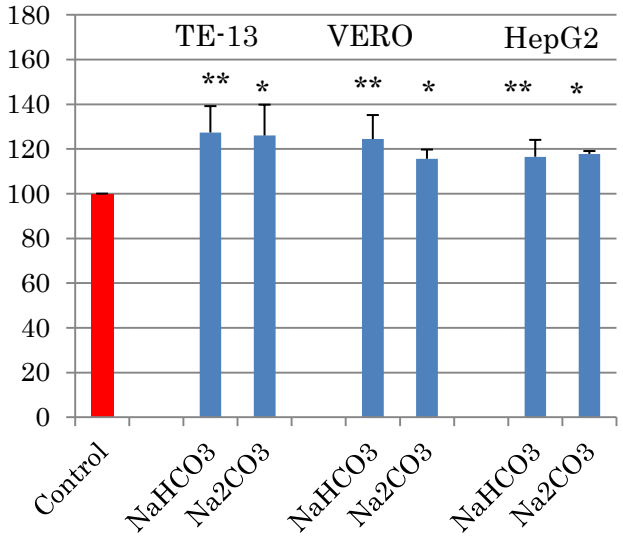
Figure 3. Insulin effect on glucose consumption. Concentrations of insulin

were 0.1, 0.5, 1.0, 5.0, 10, and 50 μg/ml. Data represent means ± SDs of six independent experiments.

Figure 4. Regression lines based on plotting of C content against G content. Nucleotide contents of complete genomes of various cellular organelles were normalized. This is a modified version of a figure in our previous article: Natural Science, 2018; 10 (9): 338-369 [49]. Large red and blue closed circles represent *Monosiga brevicollis* and *Homo sapiens* mitochondria, respectively. Vertebrate mitochondria; (asterisk), high C/G invertebrate mitochondria; (triangle), low C/G invertebrate mitochondria, (cross), bacteria; (circle), non-animal mitochondria and chloroplasts; (diamond), and chromosomes; (square).

Fig. 1

A


180
160
140
120
100
80
60
40
20
0
TE-13
VERO
HepG2
**
*
**
*
**
*
Control
NaHCO3
Na2CO3
NaHCO3
Na2CO3
NaHCO3
Na2CO3

B

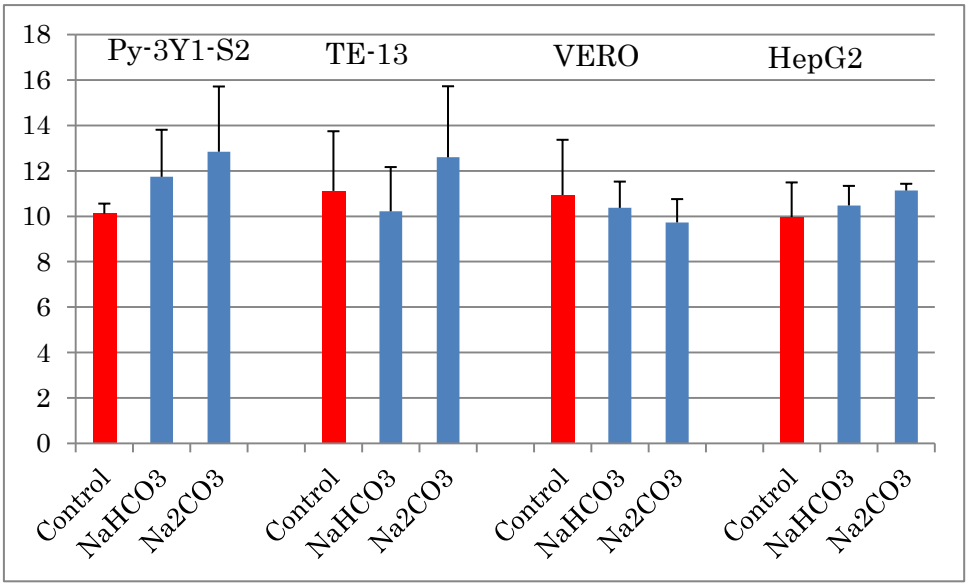

18
16
14
12
10
8
6
4
2
0
Py-3Y1-S2
TE-13
VERO
HepG2
Control
NaHCO3
Na2CO3
Control
NaHCO3
Na2CO3
Control
NaHCO3
Na2CO3
Control
NaHCO3
Na2CO3

Fig. 2

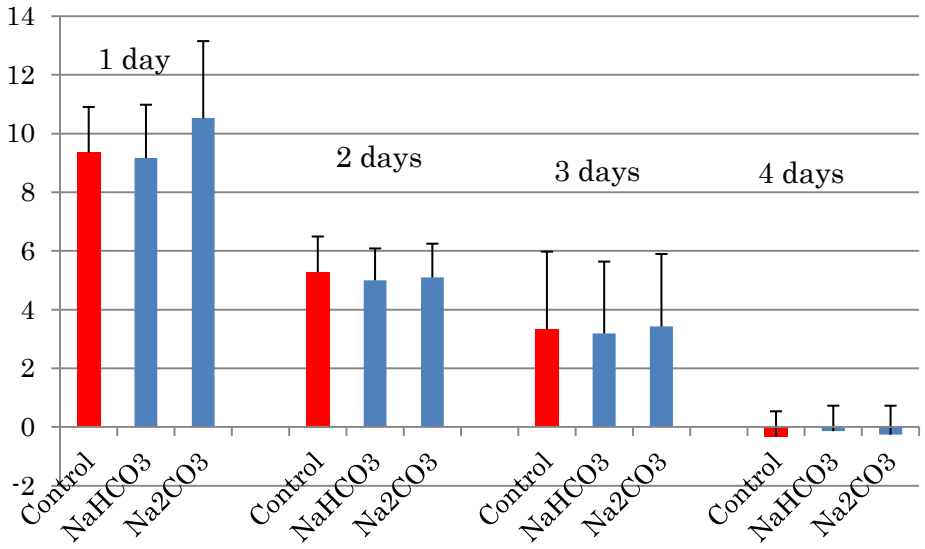

14
12
10
8
6
4
2
0
-2
1 day
2 days
3 days
4 days
Control
NaHCO3
Na2CO3
Control
NaHCO3
Na2CO3
Control
NaHCO3
Na2CO3
Control
NaHCO3
Na2CO3

Fig. 3

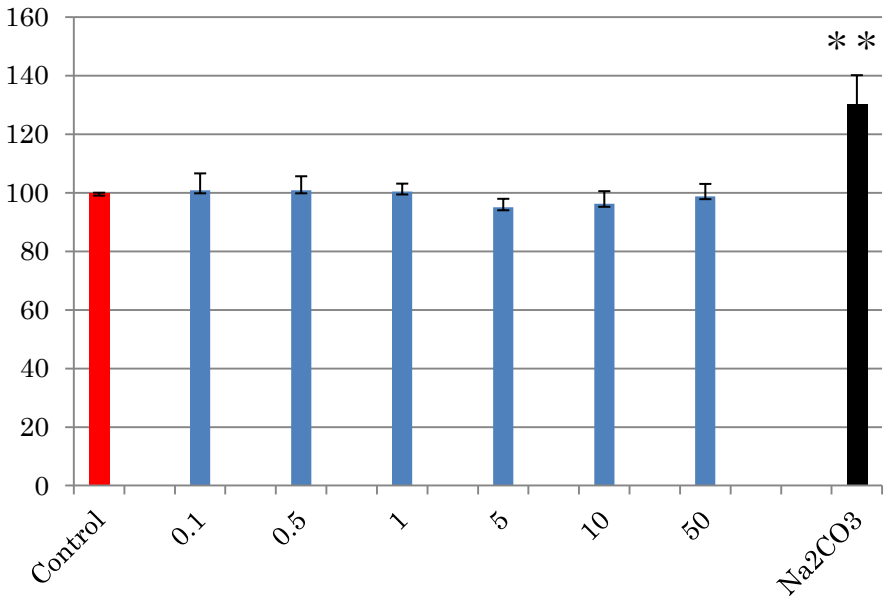
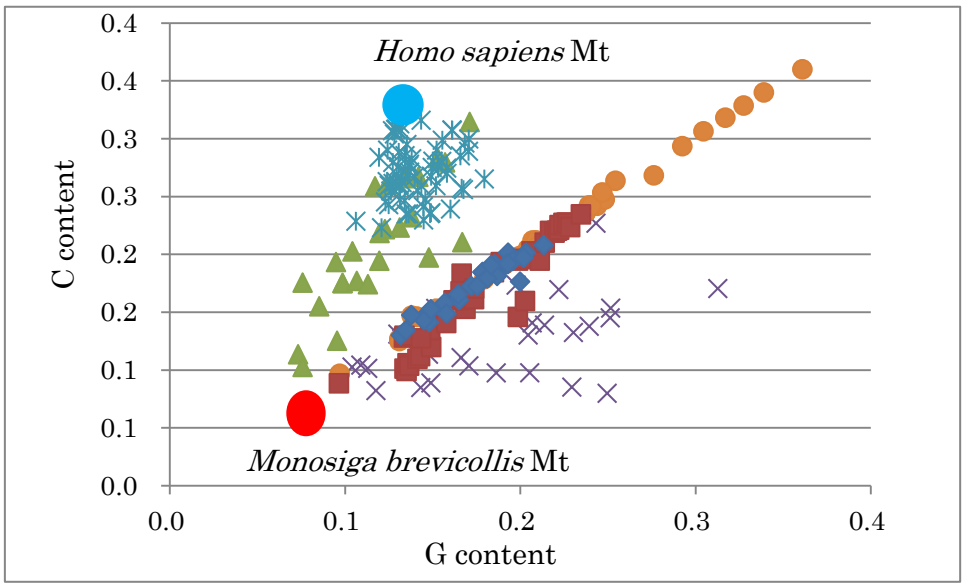


Fig. 4


0.4
0.4
0.3
0.3
0.2
0.2
0.1
0.1
0.0
C content
Homo sapiens Mt
Monosiga brevicollis Mt
0.0
0.1
0.2
0.3
0.4
G content