



PIGS IN SPACE: EFFECT OF ZERO GRAVITY AND AD LIBITUM FEEDING ON WEIGHT GAIN IN CAVIA PORCELLUS



SPACE EXES

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ABSTRACT:

One ignored benefit of space travel is a potential elimination of obesity, a chronic problem for a growing majority in many parts of the world. In theory, when an individual is in a condition of zero gravity, weight is eliminated. Indeed, in space one could conceivably follow ad libitum feeding and never even gain an gram, and the only side effect would be the need to upgrade one's stretchy pants ("exercise pants"). But because many diet schemes start as very good theories only to be found to be rather harmful, we tested our predictions with a long-term experiment in a colony of Guinea pigs (*Cavia porcellus*) maintained on the International Space Station. Individuals were housed separately and given unlimited amounts of high-calorie food pellets. Fresh fruits and vegetables were not available in space so were not offered. Every 30 days, each Guinea pig was weighed. After 5 years, we found that individuals, on average, weighed nothing. In addition to weighing nothing, no weight appeared to be gained over the duration of the protocol. If space continues to be gravity-free, and we believe that assumption is sound, we believe that sending the overweight — and those at risk for overweight — to space would be a lasting cure.

INTRODUCTION:

The current obesity epidemic started in the early 1960s with the invention and proliferation of elastane and related stretchy fibers, which released wearers from the rigid constraints of clothes and permitted monthly weight gain without the need to buy new outfits. Indeed, exercise today for hundreds of million people involve only the act of wearing stretchy pants in public, presumably because the constrictive pressure forces fat molecules to adopt a more compact tertiary structure (Xavier 1965).

Luckily, at the same time that fabrics became stretchy, the race to the moon between the United States and Russia yielded a useful fact: gravity in outer space is minimal to nonexistent. When gravity is zero, objects cease to have weight. Indeed, early astronauts and cosmonauts had to secure themselves to their ships with seat belts and sticky boots. The potential application to weight loss was noted immediately, but at the time travel to space was prohibitively expensive and thus the issue was not seriously pursued. Now, however, multiple companies are developing cheap extra-orbital travel options for normal consumers, and potential travelers are also creating news ways to pay for products and services that they cannot actually afford. Together, these factors open the possibility that moving to space could cure overweight syndrome quickly and permanently for a large number of humans.

We studied this potential by following weight gain in Guinea pigs, known on Earth as fond of ad libitum feeding. Guinea pigs were long envisioned to be the "Guinea pigs" of space research, too, so they seemed like the obvious choice. Studies on humans are of course desirable, but we feel this current study will be critical in acquiring the attention of granting agencies.

MATERIALS AND METHODS:

One hundred male and one hundred female Guinea pigs (*Cavia porcellus*) were transported to the International Space Laboratory in 2010. Each pig was housed separately and deprived of exercise wheels and fresh fruits and vegetables for 48 months. Each month, pigs were individually weighed by duct-taping them to an electronic balance sensitive to 0.0001 grams. Back on Earth, an identical cohort was similarly maintained and weighed. Data was analyzed by statistics.

RESULTS:

Mean weight of pigs in space was 0.0000 +/- 0.0002 g. Some individuals weighed less than zero, some more, but these variations were due to reaction to the duct tape, we believe, which caused them to be alarmed push briefly against the force plate in the balance. Individuals on the Earth, the control cohort, gained about 240 g/month ($p = 0.0002$). Males and females gained a similar amount of weight on Earth (no main effect of sex), and size at any point during the study was related to starting size (which was used as a covariate in the ANCOVA). Both Earth and space pigs developed substantial dewlaps (double chins) and were lethargic at the conclusion of the study.

CONCLUSIONS:

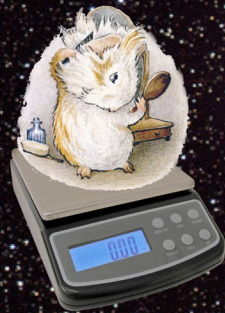
Our view that weight and weight gain would be zero in space was confirmed. Although we have not replicated this experiment on larger animals or primates, we are confident that our result would be mirrored in other model organisms. We are currently in the process of obtaining necessary human trial permissions, and should have our planned experiment initiated within 80 years, pending expedited review by local and Federal IRBs.

ACKNOWLEDGEMENTS:

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Natural Killer Cells Disintegrates In Vitro Model Tumors but not Intravital Tumors



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INTRODUCTION

Adaptively transferred E.2 activated NK cells accumulate in some tumour types after arrival at the microenvironment of the tumour. In vitro NK cells can perform heterocirculatory migration, penetrate the perivascular blood spaces and adjacent extracellular matrix, and form cellular cell-to-cell contacts with tumour cells. However the preconditions seem to be fulfilled for a complete action to take place. However, most studies demonstrate significant limitations of adoptively transferred cells that ultimately influence tumours in small and efficient effector-to-target cell ratios in early advanced. Consequently, one factor of importance determining quantitatively the influence is the possibility of the activated effector cells to traverse the vasculature. Due to matrix constituents might provide physical barriers for cellular migration. Recently, the architecture of the extracellular space in the intertumoural interstitium of the matrix is expected to create the pathway for the migrating NK cells. For example, preliminary data indicate colonies with a loose growth pattern are associated with a high degree of tumour metastasis with a dense growth pattern being established [Jernsma et al (Göteborg)]. In our tumours we have found compressed extracellular matrices supporting a culture of the extracellular matrix as a possible barrier for tumour localization of NK cells [Jernsma et al (Göteborg)]. We have therefore explored the ultrastructure morphology of the tumour interstitium.

ABSTRACT

We have re-evaluated the morphology of the infiltration process of NK cells into metastatic cell colonies, increasingly simplified experimental *in vitro* and *intra vital* models were evaluated by electron microscopy. Firstly, studying tumours grown intraperitoneally established the influence of distribution, arrival and the transvascular migration process. Secondly, in tumour cell colonies formed *in vitro* the infiltration was assumed to depend on cell-to-cell and cell-to-matrix contacts due to the lack of contribution by preformed tissue components, influencing the shape and extension of the tumour growth. Thirdly, NK cells and tumour cells were separated with the matrix equivalent (Matrigel®) in which the ability of the NK cells to affect the integrity of matrix components during formation of target cell contacts could be examined. The Matrigel® re-circulation experiments revealed that NK cells pre-cultured for 5 days altered generally the initial homogeneity texture of the Matrigel®, a monoporous appearance by 5 h became a loose fibrous network (more than 5 days) NK cells, instead formed large associations in the Matrigel®, that seemed to contain an expanding material. Also was the remaining matrix less affected with the long-term cultured NK cells. The metastatic cell colonies formed *in vitro* and analogy with Matrigel® experiments, long-term cultured NK cells were located in wide spaces between tumour cells. After adaptive intraperitoneal injection, isolated NK cells had infiltrated the tumour mass and were generally surrounded by a slightly wider interstitial space than the adjacent tumour cells.

It is suggested that the compressive capacity of NK cells to disintegrate extracellular matrix, and dissolve tumour equivalents *in vitro*, takes with effects by proteases. Similar effects was not confirmed in the more complex *intra peritoneal* model, possibly due to presence of protease inhibitors or restraints by endogenous extracellular matrix components.

RESULTS

Fig 1. Electron micrographs showing the structural alterations of the Matrigel after 1 h (A) and 24 h (B and C) of co-culture with foreign cell (foreign cells) (NK cells) (Fig. 1, A, B, C). Adaptively transferred NK cell is surrounded by a thicker network of extracellular matrix (Bar = 10 µm). Only areas spread around (arrowheads) of foreign material after 24 h (except in case of association with non-tumour cells). These areas are located in a matrix layer of other homogeneous texture (arrow) (Bar = 10 µm).

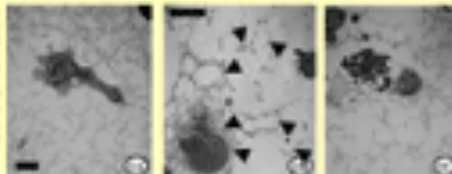


Fig 2. Electron micrographs of Matrigel sheets after 5 h co-culture with day 5 NK cells and B16 melanoma cells. A, An NK cell that is partly surrounded by Matrigel. Multiple cellular projections penetrate into the matrix extracellular matrix. An elongated empty cavity penetrates in the matrix outside from the cell, giving the impression that the cell has negatively its position during digestion of the Matrigel. Bar = 10 µm. B and C, NK cell associated to large cavities in the Matrigel and a melanoma cell (B) that is closely associated to a layer of homogeneous matrix. Note that the matrix material surrounding the cavity has a denser structure giving a compressed appearance (between arrows). This fibrous structure in the interstitial cell also has a higher density, while the bulk of the extracellular material in the face of view is slightly swollen (lower cytoplasmic spaces in NK cell represent glycogen stores. Bar = 10 µm).

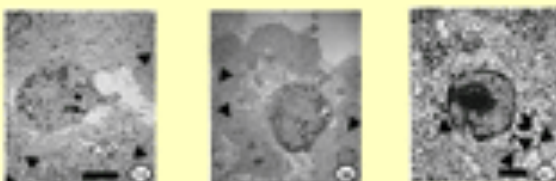


Fig 3. NK cells that have infiltrated melanoma tumour formed *in vitro* (A and B) and *in vivo* (C, D and E). In the *in vitro* model the foreign cell clasts aggregates in the expanding day 5 NK cell is situated in a wide interstitial space. Note that adjacent melanoma cells are closely apposed along interstitial extracellular matrix (arrows. Bar = 5 µm. E, In the *intra peritoneal* tumour a day 5 NK cell is associated into a narrow space. The cell lacks cytoplasmic filamentous structure by granules with dense granules or vacuolated contents (dark areas). Its contour is highly irregular and along part of its circumference the related to the melanoma cells is slightly wider and contains a homogeneous material of increased electron density (arrowheads). Bar = 2 µm.

DISCUSSION

NK cells *in vitro* change functional and morphological properties around the 4th day of culture. The younger cells develop numerous characteristic morphological peculiarities and an highly cytotoxic in ¹²⁵I-thymidine assays whereas the development after day 5 into large elongated spindle cells is concordant with a decline in cytotoxic capacity, a transformation effect on tumour cell colonies (Eggen et al (Oslo) (J Immunol 1987;138:3523)) and a release of fact around tumour (Sankilay et al (London) Int J Cancer 1995;57:285). Release and proteolytic action of matrix might therefore have contributed to the general degradation of Matrigel that was observed by NK cells up to day 5 of culture age. This also suggested that the relatively unmodified microclimate in the matrix material that was seen around older NK cells is related to a retention of an expanding extracellular material. The mentioned fact might suggest that a release of proteases is required to have a complex composition including proteolytic such as matrix metalloproteinases. Thus, the hydrolytic properties of such extracellular constituents could readily explain the images of expanding, Matrigel-compressing zones around the NK cells. However, proteolysis (or hyaluronidase) for also observed a slight although to a lesser degree are known to find MMPs (7) and Stromelysin, (J Biol Chem 2002;277:4193) providing an explanation to the lack of a general effect on the Matrigel architecture in these experiments. In large scale also experimental data point to 1-6 NK cells were located at the end of elongated surface Matrigel associations.

Taken together, the probability that NK cells acquire matrix degrading enzymes, and that loose synthesis and release of proteases, support a hypothetical contention that unless a functional form these NK cell action. Without release of proteases the retention of MMPs gives rise to a general loosening of the matrix extracellular matrix, whereas the association of MMPs give rise to cellular proteolysis might provide a mechanism which the matrix degradation is retained, or even delayed, in the immediate vicinity of the cell as proposed also by Tu and Hoshino. Current reports on the interaction between the proteolysis domain of metalloproteinase MMP-2 and proteolytic active MMP-1 are in agreement with such observations (Tu and Hoshino, *Cancer Res* 1998;58:3852). Further, the similar pattern with NK cells in cultured spaces within the matrix interstitium is suggested to reflect a dual action from matrix degradation by proteases and a physicochemical of the extracellular space by moved material.

However, the intratumoral tumours revealed largely the possible tumour deintegrating effect of NK cell infiltration. In the results from the *in vivo* tumours cannot be extrapolated to the interstitial matrix. Whether or not these NK cell actions on matrix material and simplified tumour models would affect a potentially beneficial effect on established tumours is also unclear for determination of progress. If so, there would be a lack of insight on how to optimize NK cell distribution so that the wide of interstitial will be achieved and if a penetration of matrix lymphocytes with tumour infiltrating capacity into the vascular matrix.

MATERIAL and METHODS

Adoptive NK cells were prepared by spleen homogenization of mice (C57BL/6) mice and cultured in RPMI cell medium with supplements and viable tumour components. In day 5, co-cultures were prepared and the liquid were gently shaken and processed (20% (v/v) serum cells) normally placed in the plate surface. Non-adherent cells were removed by washing the plate with RPMI. The adherent cells were harvested into a 15 ml centrifuge tube and centrifuged at 400g for 5 min. The supernatant was removed and the cells were washed with RPMI. The cells were then cultured in RPMI cell medium supplemented with 10% fetal calf serum. Media was harvested after 5 days of culture and cells were harvested for analysis. For *in vitro* co-culture of NK cells and B16 melanoma cells, 10⁶ B16 melanoma cells were cultured for 5 days in RPMI cell medium. The supernatant was harvested and cells were washed with RPMI. The cells were then cultured in RPMI cell medium supplemented with 10% fetal calf serum. Media was harvested after 5 days of culture and cells were harvested for analysis. For *in vivo* co-culture of NK cells and B16 melanoma cells, 10⁶ B16 melanoma cells were cultured for 5 days in RPMI cell medium. The supernatant was harvested and cells were washed with RPMI. The cells were then cultured in RPMI cell medium supplemented with 10% fetal calf serum. Media was harvested after 5 days of culture and cells were harvested for analysis.

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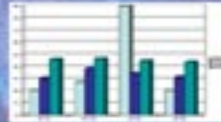
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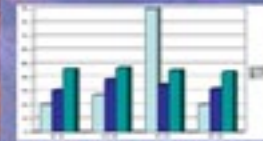
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