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Comparison of Cooling Systems During Islet Purification

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Islet isolation is a complex procedure that includes digestion and purification of pancreatic tissue. As we move towards clinical regulatory control and standardization, understanding of the detailed stages of the procedure have become increasingly important. Purification on a COBE 2991 density gradient allows human islets to be separated from a large volume of acinar tissue. Cooling the gradient and tissue is thought to be important to reduce metabolic activity but cooling systems for the gradient are expensive, with limited availability. In this study, the efficiency of cooling methods for the COBE 2991 cell separator has been investigated. The two cooling systems were: a) COBE 2991 modified internally to allow coolant (polyethylene glycol) from a chiller to circulate either side of the spindle and around the bowl (original system), and b) an air-cooled system using an air conditioner to blow cold air into the bowl from above (air cooler system). Cooling required 20 min for the original system and temperature was stabilized within 4–7°C. The air system cooled rapidly but was not stable. There was an increase in the temperature of the medium with using both systems during centrifugation because of heat generated by the COBE machine; however, the temperature of the medium after centrifugation with the air system was significantly higher than that with the original system (13.3 ± 0.2°C vs. 8.7 ± 0.7°C, $p < 0.05$). The original cooler system was found to be more efficient at reducing heat generated by the COBE machine than the air system. Further investigation of the importance of the recorded temperatures is required.

Key words: Density gradient; Islet purification; Coolant; Chiller; Temperature

INTRODUCTION

Several islet transplantation programs are initiated worldwide following the reports of successful islet transplantation (11). Although islet transplantation has been demonstrated as a relatively easy and safe procedure for treatment of type 1 diabetes, the islet isolation procedure is complex and requires a great deal of experience to yield sufficient viable islets that can be transplanted into diabetic patients. Current isolation methods include the mechanically enhanced enzymatic digestion of the pancreas followed by the purification of islets using a density gradient centrifugation. Purification of large numbers of islets has advanced rapidly with the introduction of a cell processor (COBE 2991; COBE Laboratories, Inc., Lakewood, CO) and continuous density gradients (6,9).

Failure in obtaining high purity of islets generally results from a decrease in density of acinar tissue. Release of endogenous enzymes from the intact and disrupted acinar cells has the potential to cause tissue damage and to decrease acinar tissue density. Cytoprotective strategies, such as a reduction in temperature during gradient centrifugation, have been applied in order to maintain or enhance the difference in density between islet and acinar tissue. The importance of temperature of the gradient media has been highlighted for islet purification. A comparison of bovine serum albumin-based gradients at 4°C and 22°C showed no difference in the relative density of human islets and acinar tissue, although greater efficiency was seen with pig islet purification at 4°C (2). Separation of rat islets on discontinuous gradients at 4°C was found to lead to significantly more islets than at room temperature (13). On the other hand, Rilo et al. found no difference in number, purity, or viability of human islets separated on Euro-Ficoll gradients at 4°C or 24°C, and they did observe morphological evidence of fragmentation and degranulation in those separated at 4°C (8).

There is an argument for cold temperature gradients and efforts have been made to cool the gradient media during islet purification. A small number of COBE 2991s were modified at the end of the 1980s to accommodate a chiller system to cool the gradient media to 4°C, with

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the aim of improving islet separation and reducing cell damage. Until recently such chiller modifications were not generally available. This has led to a number of alternative purification protocols, including a use of COBE in a refrigerated room (5) and a fast loading method with nonrefrigerated COBE (Dr. Markmann, Philadelphia, personnel communication). Such techniques need to be validated by successful clinical islet transplantation but this is difficult in terms of the multivariate nature of the isolation process. Few centers have access to more than one chiller modification. In the current effort to reproduce the clinical success of the Edmonton islet transplants (11) by adoption of their isolation procedures, there is a need to revisit the cell separation and purification methods, particularly with regard to temperature. In this study we have taken detailed temperature measurements for the coolant and media throughout the gradient-loading and unloading procedure to compare the effectiveness of different cooling systems.

MATERIALS AND METHODS

Gradient Preparation

The method of gradient preparation used routinely in Edmonton for human islet isolation was used in this study except where indicated. This was a top loaded continuous density gradient. The gradient media were removed from the fridge into the laminar flow cabinet and inserted into a cooled metal block kept in the −20°C freezer before use. The equivalent of digest layers was placed on ice. The centrifuge bowl of the COBE was spun at maximum speed and the pressure overload tested. After insertion of a COBE bag, 150 ml of high-density (1.100 g/cm^3) Biocoll (Biochrom, Berlin, Germany) was loaded into the bag, air was released, and the machine stopped to allow the hydraulic system to return to basal level. Then 130 ml of high density and 140 ml of low density (1.077 g/cm^3) were added via the gradient maker to create a continuous density gradient at the centrifuge speed of 2500 rpm. The digest layer equivalent was added as 100 ml of University of Wisconsin solution (UW), and 50 ml of capping layer (MEM), all at a pump rate of 55 ml/min. After 5-min timed spin, the gradient was collected into 6×250-ml bottles with the super-out function at a rate of 100 ml/min. Each stage of the procedure was timed to ensure reproducibility.

Cooling Systems

Two cooling systems were tested in a high-efficiency particulate air filtered islet isolation laboratory at room temperature of 18°C as follows. In addition to these two systems, an unmodified COBE was tested in rooms at 4°C and 18°C.

Original Cooler System. The COBE 2991 has a hydraulic system to facilitate the removal of the gradient via the super-out function. This system comprises fluid, sited in a bag on a shelf inside the machine, pumped up the centrifuge spindle into a space between the centrifuge bowl and flexible membrane and back down a metal sheath surrounding the spindle. The cooling system comprised a metal bowl, with a hollow compartment, underneath and surrounding the centrifuge bowl. This is connected via a tube with two hollow compartments around the centrifuge spindle. Tubing from the hollow tube to the inlet and outlet of a recirculating chiller (PolyScience, Chicago, IL) allows cold polyethylene glycol (1:3 in water) to be pumped alongside the spindle into the outer bowl and out again down the center of the spindle into the chiller for cooling and recycling.

Air-Cooled System. An industrial grade air conditioner unit (cooler) was connected to holes cut in the perspex lid over the centrifuge bowl via wide hoses and tumble drier connectors. Cold air was blown into the machine and out from the sides of the perspex lid into the room. Warm exhaust from the air cooler was directed to the outflow from the clean room. The air cooler was an MC20LT/2MOD (Broughton, UK) with an integral fan delivering 20,000 BTUs of cooled air, in which the thermostat is overridden before use (Fig. 1).

Measurement of Temperature

Temperatures were recorded using temperature probes connected to a monitor (Mon-a therm; Mallinckrodt Inc., St. Louis, MO) or using a laser thermometer (Testo Quick temp 826-T1; Testo Ltd., Alton, UK). Probes were sited at 1) inlet to COBE from chiller, 2) outlet

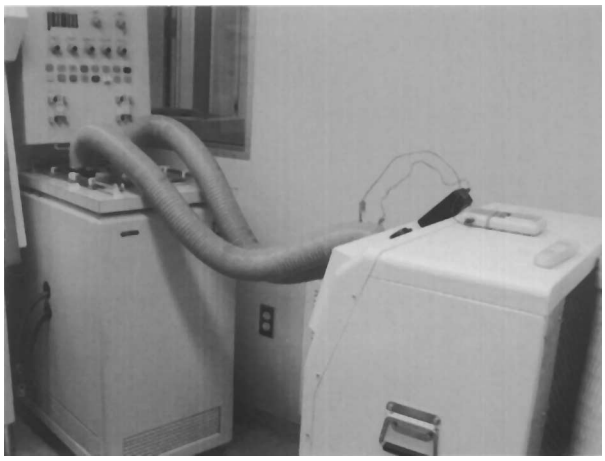

Figure Air-cooled COBE 2991 with Broughton air conditioner connected to the perspex lid above the centrifuge bowl with wide hoses containing temperature probes.

from COBE to chiller, 3) inside the tubing from the gradient maker as it connects to the COBE bag tubing, and 4) inside the collection tubing as it connects from the COBE bag tubing.

Statistical Analysis

Statistical analysis was performed using Microsoft Excel software and unpaired Student's *t*-test. Data were considered statistically significant for values of $p < 0.05$.

RESULTS

Stability of the Cooler Systems Without COBE Operation

In the original cooling system, temperatures at both inlet to and outlet from COBE dropped below 5°C at 20 min after initiation of the cooling system. Thereafter temperatures were stabilized at 4°C. The cooling pattern of the air cooler system was found to differ from the original and modified systems. There was a programmed 5-min delay before the compressor started but then rapid cooling occurred within 5 min to below 4°C. The cold air from the cooler was relatively stable between 10 and 25 min and then, as the compressor stopped and started (a feature of the machine), the temperature dropped slightly further to below 0°C.

Temperature Changes in the Cooler System During Procedure

The next task was to monitor the temperature of coolant while using the COBE with the two cooling systems. The cooler system was activated 20 min in advance of gradient loading to ensure stability based on the findings above. During the procedure with the original system, the temperature of the polyethylene glycol inflow to the COBE was stable at 4°C, which was similar to the temperature when measured without COBE operation (data not shown). The outflow temperature was stable at 4°C, and after initiation of loading gradient it slightly increased to 7°C (Fig. 2a). In the air cooler system, the variation recorded was reflected in both the inflow and outflow (Fig. 2b). The cold air leaving the COBE mirrored the inflow cooling pattern but absorbed considerable heat from the machine before being recycled to the air cooler. The peak temperature in outflow coincided with the opening of the centrifuge bowl to add the bag and rapid influx of room temperature air.

Temperature of Medium During Centrifugation

It was not possible to directly record the temperature of the medium during centrifugation so, as an alternative, temperatures before and after centrifugation were recorded. The last temperature recorded as the medium entered the COBE was plotted together with the temperature as it left the COBE tubing (Fig. 3). There was an increase to 8.7 ± 0.7°C with the original cooler system and to 13.3 ± 0.2°C with the air-cooled system ($p < 0.05$). The noncooled COBE showed the largest increase in temperature to 17.4 ± 1.0°C ($p < 0.05$ vs. original system). However, placing COBE in a refrigerated room did not show a significant difference in temperature after centrifugation (9.4 ± 2.8°C) compared to the original system.

DISCUSSION

Development of the purification system for human islets occurred more than a decade ago (7). The transfer of the procedure to a more routine clinical environment and the introduction of regulation and guidelines following publication of the first Edmonton results (11) and subsequent ITN trial (12) has encouraged us to revisit the use of the COBE 2991 for islet purification and to study this critical part of the procedure in more depth.

The lack of availability of cooler systems for the COBE 2991 has also led to the use of cold rooms to house the machine (5) or fast loaded gradients at room temperature (Dr. Markmann, Philadelphia, personnel communication). The large series of successful clinical islet transplants from Edmonton have used the original and modified design of cooled COBE to maintain the gradient and digested pancreatic tissue as close to 4°C as possible (10). More recently, Hering et al. have reported clinical reversal of diabetes using a similar method but incorporating a different gradient medium (iodixanol based) used on a COBE 2991 housed in a cold room (4).

Comparison of the original and air-cooled systems showed the original system to be stable and efficient in lowering temperature while the air-cooled system, although producing air below the temperature required, was less stable and resulted in gradient temperatures midway between the original system and no cooling. The variation in temperatures shown in Figure 2b appeared to be due to intermittent cut-out of the compressor. It should be noted that the difference between inflow and outflow was an indication of the heat being absorbed within the machine.

Fluctuation of temperature during operation of the COBE 2991 showed an increase in temperature (from 4°C to 7°C) during the super-out phase as shown in Figure 2a (original cooling method outflow from COBE). This increase appeared to be due to the movement of hydraulic fluid (at ambient temperature) up into the bowl in order to expel air (after loading high-density medium or the gradient after centrifugation). The original cooling system has the advantage that although the hydraulic fluid is at room temperature and is warmed by the mechanical workings of the machine, it is cooled as

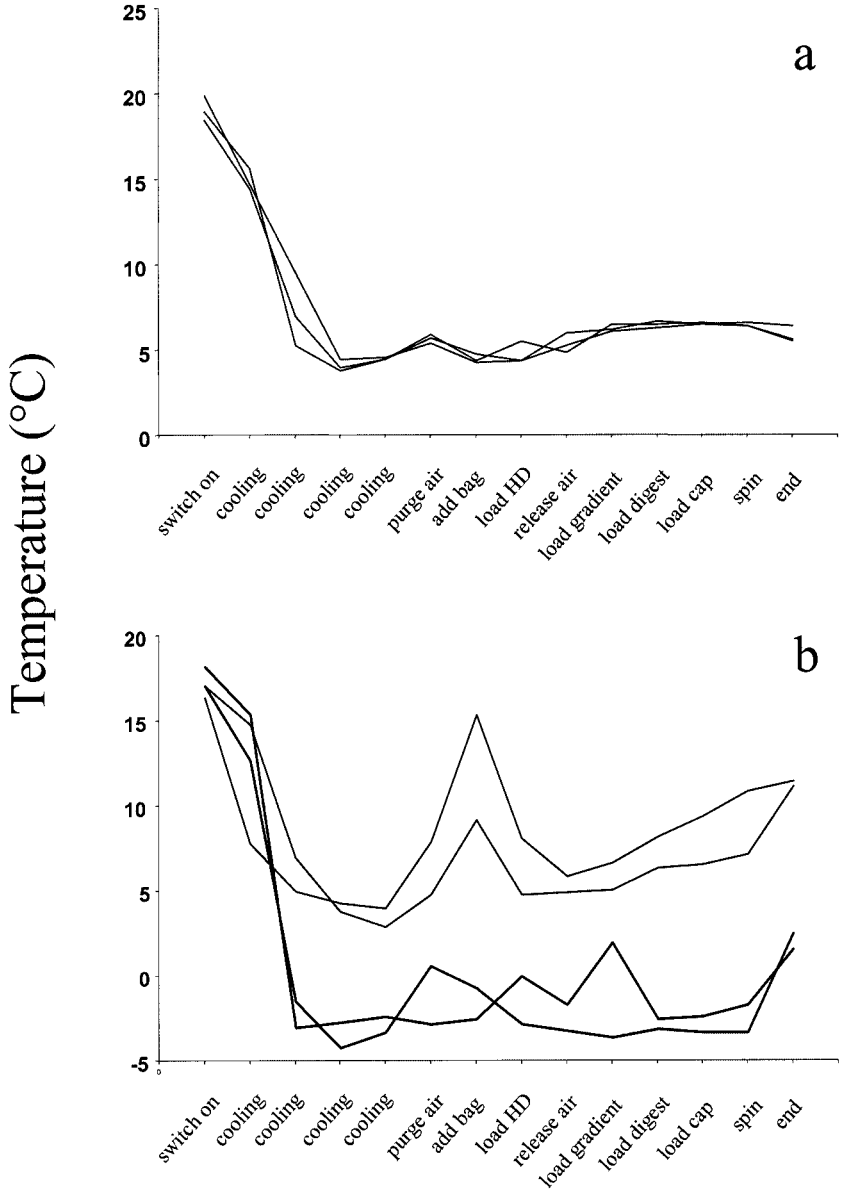

Figure 2. Temperature changes of the coolants during COBE 2991 use. Temperatures of the coolants were measured at outlet from COBE to chiller in the original system (a) ($n = 3$). For the air cooler system, temperatures at both inlet to (thicker line) and outlet from COBE were plotted (b) ($n = 2$). HD, heavy density.

it passes through the precooled spindle. In contrast, the air-cooled system cools the bowl from above and does not cool the fluid in the spindle, leaving the gradient prone to increase significantly above the loading temperature.

Measurement of the temperature of the gradient inside the bag was not possible but has been represented by the loading and collection of the media immediately pre- and postcentrifugation. This is a critical period that represents the changes in temperatures of the digest and is shown for all systems in Figure 3. Attempts to precool the gradient maker or COBE bag (in the fridge or freezer) before use were found to have less impact on the collection temperature than the storage temperature of the media, particularly for the air-cooled and noncooled system (data not shown).

The implication of the changes in temperature during islet purification has not been addressed by this study; however, temperature has been considered to be an im-

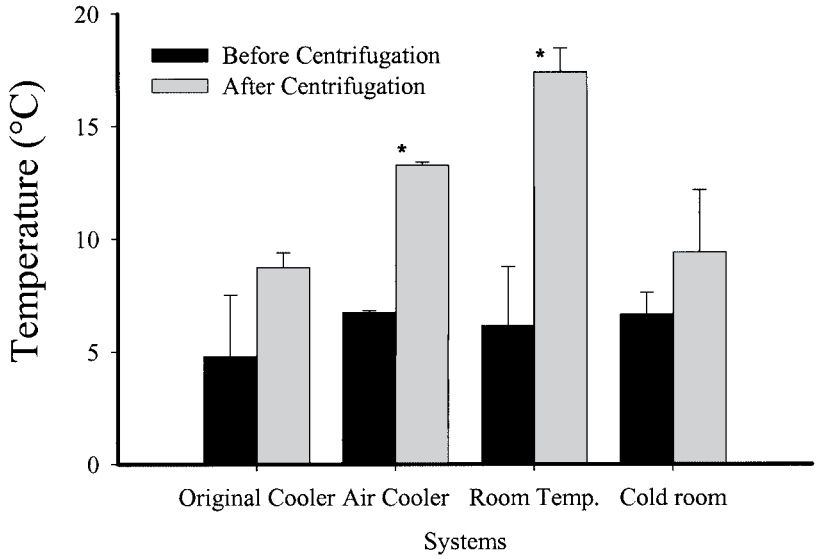

Figure 3. Increase in temperature of media during centrifugation. Temperatures of medium measured precentrifugation and postcentrifugation are presented for each cooling system ($n = 3$). *$p <$ 0.05 versus original system.

portant part of purification. The temperature of UW is known to affect tissue and cellular function, and the use of this medium in the gradient suggests that increases in temperature above 4°C may have detrimental effects on the cells (1,3).

It is clear that even making considerable effort to keep the digest and islets at a steady 4°C there are variations in temperature that are unavoidable. Does this matter? Having established that variation in temperature exists, the relative importance of temperature and the degree of variation that can be tolerated by human islets during purification without functional damage needs to be investigated further. Fluctuations recorded in this study lead naturally to consideration of temperatures throughout the process as a whole, and accurate determinations of the actual rather than presumed temperatures are desirable. In a process that shows variable outcome and relies to a certain extent on the technical skill of the personnel involved, temperature level and control may be critical factors.

In conclusion, we investigated the temperatures of cooling systems for the COBE 2991 and found that the original cooling system is more efficient at reducing heat generated by the COBE machine than the air system. Housing the COBE machine in the cold room may provide an alternative to the original cooling system. Further investigation of the importance of the recorded temperatures is required.

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