Acute insulin response (AIR): review of protocols and clinical interest in islet transplantation

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SUMMARY
Various stimuli have been used in clinical practice to test islet function, including intravenous glucose, arginine — both at basal glucose levels and with the hyperglycaemic clamp, tolbutamide, glucagon and glucagon-like peptide 1. The subsequent first phase insulin response (also termed acute insulin response or AIR) to intravenous glucose or arginine has been quantified in a variety of ways, from the mean serum insulin measured at multiple times after glucose injection to the mean value above baseline of serum insulin at 2 to 10 min. The purpose of this study was to review the different protocols of AIR calculation and their pitfalls, and to assess the results of AIR in the islet transplantation field. By investigating the first phase of insulin secretion, AIR provides both a qualitative and a quantitative approach to insulin secretion. In conclusion, AIR could help to predict the need for a second or third islet injection in islet transplantation. These specific indications, however, need to be confirmed by future studies and completed by other approaches such as insulin sensitivity studies and in vivo morphological assessment of islet mass.

Key-words: Acute insulin response · Islet transplantation · AIR post glucose · AIR post arginine.

RéSUMÉ
La réponse insulinique aiguë (AIR) : intérêt clinique en transplantation insulaire
Divers stimuli sont utilisés en clinique pour évaluer la fonction des îlots β pancréatiques, notamment le glucose intraveineux et l’arginine - à la fois à l’état basal et au cours de clamps hyperglycémiques- ainsi que le tolbutamide, le glucagon et le glucagon-like peptide 1. La première phase de réponse insulinique à une injection intraveineuse de glucose ou d’arginine (aussi appelée réponse insulinique aiguë ou AIR) a été calculée par des formules variées, allant de la moyenne des insulinsémies mesurées à différents temps après une injection de glucose à la moyenne des insulinsémies supérieures à l’insulinsémie basale 2 à 10 minutes après stimulation. Le but de cette revue était 1) de revoir les différents protocoles utilisés pour le calcul de l’AIR ainsi que leurs écueils 2) d’étudier plus particulièrement l’intérêt de l’AIR dans le domaine de la transplantation insulaire. En explorant la première phase de sécrétion de l’insuline, l’AIR permet une approche qualitative et quantitative de l’insulinosécrétion. En matière de greffe d’îlots, l’AIR après glucose pourrait prédire la survie des îlots c’est-à-dire le pronostic de la greffe, tandis que l’AIR après arginine serait mieux corrélée à la masse de cellules β transplantées, guidant ainsi l’indication d’une nouvelle greffe. L’utilisation de l’arginine permet d’éviter la glucotoxicité liée à l’hyperglycémie après glucose. En conclusion, l’AIR présente un intérêt en transplantation insulaire et chaque stimulus semble trouver des indications spécifiques. Des études complémentaires sont nécessaires pour les confirmer tandis que d’autres approches telles que l’évaluation de l’insulinosensibilité et les techniques morphologique in vivo de la masse insulaire greffée seraient utiles pour compléter ces informations.

Mots-clés : Réponse insulinique aiguë (AIR) · Greffe d’îlots pancréatiques · AIR après glucose · AIR après arginine.


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Introduction

Islet transplantation is proposed to patients using insulin-based regimens who are unable to achieve satisfactory glucose control without frequent episodes of serious hypoglycaemia. We now know that this approach is both reproducible and successful [1-3]. The major question that remains is “how long will the transplanted islets survive?” A recent study has demonstrated that even if islet-transplanted patients remain C-peptide positive for as long as 5 years, the rate of insulin independent patients decreases over time [4]. These long-term islet transplantation results depend on several parameters, including insulin sensitivity and beta cell function.

While the in vivo effects of insulin can be directly measured with a variety of techniques, beta cell function proves more complex to assess [5-7]. The most informative intravenous means of testing beta cell function (hyperglycaemic clamp, graded glucose infusions) are quite elaborate and their application is consequently limited. The AIR, i.e., the insulin concentration observed immediately after a stimulus has often been used in reason of its simplicity. Different stimuli may be used to assess it: glucose (as in the Intra Venous Glucose Tolerance Test or IVGTT), arginine, or glucose-potentiated arginine (as in the glucose-potentiated arginine insulin stimulation test or GPAIS). Despite the fact that the release of insulin after an intravenous glucose bolus (AIRg) is mediated by some but not all the cellular mechanisms that mediate insulin release during a meal, AIR had been frequently used in various fields, especially in prediction of type 1 diabetes and pathophysiological studies of type 2 diabetes [7]. In the field of whole pancreas transplantation, AIR has given rise to a particular interest. Recent data collected after long-term pancreas transplantation has demonstrated: – the normal relationship between AIRg and fasting glucose levels despite systemic venous drainage of allografts and subsequent elevated serum insulin, – a combined decrease of AIRg and AIRa as fasting glucose levels rise, – the strong predictive value of AIRg and AIR post-arginine (AIRa) regarding beta cell mass and insulin secretory reserve and – a time-related decline in beta cell function among long-term pancreas transplanted patients [8].

The aim of this review was first, to list the different protocols of AIR calculation and determine their pitfalls, and second, to summarize the knowledge drawn from AIR studies in the islet transplantation field.

Acute insulin response

Kinetics of insulin response

When given as a bolus, intravenous glucose triggers a variable multiphasic secretory response, in which one or two secondary peaks with their corresponding plasma glucose peaks can often be discerned [5]. For simplicity’s sake, only the first burst of insulin release, called “acute insulin response” or AIR, is usually considered. Its quantification has varied from the average serum insulin over time (1, 3, 5, 7, 9, 10 min after glucose injection) to the mean increment above baseline of serum insulin values between 2 and 10 min after glucose injection. When consecutive hyperglycaemic clamps are performed, there is a progressive loss of AIR, whereas the second phase insulin secretion is unaffected. The first phase is a very quick one (4 to 10 minutes), reflecting the release of the mobilizable pool of insulin. It represents only a low proportion of the total insulin pool (0.3 to 0.7%). The second phase corresponds to a slower-to-mobilize pool. Most of the signals involved in insulin secretion seem to control this second phase [9].

Cellular mechanisms of the insulin secretory response to arginine

While glucose-induced insulin secretion pathways are relatively well-known, mechanisms of arginine action are still unclear. Once arginine is injected intravenously, it enters beta cells through a cationic amino-acid transporter (CAT 2A), so that the excess of positive charges induces membrane depolarization and subsequent calcium influx and insulin release [10-12]. Arginine metabolism in islets is not involved in the insulino tropic response [13]. The differences between cellular mechanisms of glucose or arginine-induced insulin secretion create variable responses to both stimuli, clinical significance of which has to be understood.

The question is “how well does AIR represent β cell insulin release in different circumstances?”.

AIR is a simple parameter, but should be used with circumspection

Peripheral insulin assays depend on β cell release, hepatic extraction and clearance. All of these processes must be considered when judging beta cell function from systemic insulin concentration. It is also important to point out that AIR may describe β cell secretion (or post-hepatic appearance), but not beta cell function. Indeed, assessing β cell function in vivo requires taking into account: – the insulin secretion in close relationship with blood glucose and insulin sensitivity: there is a hyperbolic relation between beta cell function and insulin sensitivity [14], and a defect in beta cell function may only be appreciated if the AIR is paired with insulin sensitivity levels, – the kinetics of insulin response after stimulation, – the multiple metabolic, hormonal and neurological influences that affect insulin secretion.
Protocols of air

IVGTT

Methods

The great heterogeneity among IVGTT protocols has already been pointed out [15]. The main divergences are related to the number of catheters used for glucose injection and sampling, the basal time (T -10 or -5 min and T0 beginning at the start or the end of the glucose injection), the glucose injection (duration of injection varying between 1 and 3 min±15 seconds), the concentration of glucose used (20 to 66%), the dose of glucose injected (0.5 g/kg in most studies, 0.3 in others), the mention of a maximal dose of glucose given (no maximal dose in some studies, 20 to 50 g of glucose in others). Blood arterialization is sometimes performed. And finally, different commercially available kits are used to measure serum insulin levels.

Each of these parameters may influence the results [16-19]. In clinical practice, an input greater than 7 g/min with a glucose dose greater or equal to 20 g seems to be the best criterion to reach maximal stimulation of insulin secretion [16,17]. An example of a currently used protocol (used in Edmonton) is given in table I [15].

Analysis

Different methods of calculation of AIRg are listed in table II [5]. Their effect on reproducibility and quality of insulin response has not been extensively studied. The inter-individual coefficient of variation (CV) seems high (about 56%), regardless of the study [18-21]. In contrast, the intra-individual CV is associated with divergences according to the size of the groups studied (8 to 29 healthy subjects), the duration of periods separating the two tests (2 weeks [18,22] to several months [19-21]), and the arterialization of samples [18]. Nevertheless, by comparing the average figures for IVGTT-generated parameters for insulin secretion of the various groups, Colman found that analyses of IVGTT were all similarly reproducible [22].

Arginine test (table III)

There is no complete standardization of arginine test protocols, even if they are slightly less divergent than IVGTT. Most of the time, the dose of arginine HCl administered is 5 g, though it may sometimes be adjusted to weight (0.125 g/kg). Duration of injection varies from 30 seconds (in most cases) to 1 minute. Pre-test conditioning is similar to that of IVGTT. Sampling times are T -5 or -10, T0, +2, +3, +4, +5, +6, +7, +10 min. The AIRa is computed in a variety of ways: 1) mean serum insulin at 2, 3, 4, and 5 min minus basal serum insulin [23], 2) average of the three highest serum insulins found in the samples at 2, 3, 4, and 5 min minus basal serum insulin [24] or 3) sum of serum insulins at 1 and 3 minutes [7]. The reproducibility of this test seems acceptable with an intra individual CV ranging from 9 to 27% [23].

GPAIS

This method was first described by Ward [25] in type 2 diabetes. Its principle is to potentiate the AIR obtained after

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**Table I**

An example: Protocol for IVGTT in Edmonton studies.

<table>
<thead>
<tr>
<th>Preparation before test</th>
<th>Carbohydrate intake &gt; 150 g/day three days before the test</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Usual physical activity</td>
</tr>
<tr>
<td>Fasting</td>
<td>&gt;10 h but &lt;16 h</td>
</tr>
<tr>
<td></td>
<td>– smoking discontinuation</td>
</tr>
<tr>
<td></td>
<td>– normal water intake</td>
</tr>
<tr>
<td>Test starting</td>
<td>between 7h30 and 10h00</td>
</tr>
<tr>
<td>Glucose dose</td>
<td>0.3 g/kg</td>
</tr>
<tr>
<td>Glucose concentration</td>
<td>50%</td>
</tr>
<tr>
<td>Infusion route</td>
<td>Manual or electronic system</td>
</tr>
<tr>
<td>Infusion duration</td>
<td>1 min</td>
</tr>
<tr>
<td>Time 0</td>
<td>Start of glucose infusion</td>
</tr>
<tr>
<td>Minimum number of sampling</td>
<td>T-10, T0</td>
</tr>
<tr>
<td>Catheter</td>
<td>One is enough but rinse after glucose infusion and discard dead volume before beginning sampling</td>
</tr>
<tr>
<td></td>
<td>Two is better</td>
</tr>
<tr>
<td>Calculation of AIRg</td>
<td>Mean of the insulin level at 3, 4 and 5 min after the infusion minus the mean basal insulin level</td>
</tr>
</tbody>
</table>

Note that sample timing may be shortened to 10 minutes when only AIR calculation is required.
arginine boluses (5 g) with progressively higher short hyperglycaemic plateaus. The first step of the test requires an arginine injection at euglycaemia, then a second arginine injection during clamp glycaemia at 230 mg/dL, and then at 600 mg/dL [25] (table IV). The AIR is calculated in the same way as for the arginine-alone test. Nevertheless, different glucose potentiation protocols exist. For example, five arginine stimulation tests have been implemented at different levels of serum glucose [26]: baseline, continuous infusion of glucose at rates of 300, 600, 900 mg/min, and a final infusion at a variable rate to bring the serum glucose to 27.8 mM. The maximal AIR (AIRmax) was defined as the secretory response measured at a serum glucose >27.8mM. It is a mixed estimate of sensitivity and responsiveness of beta cell function.

Table II
Different methods of AIR insulin secretion calculation have been published; Maximal insulin level, Serum insulin at a predetermined time, Ratio AUC0-10 min/ Glycaemia, Ratio increase of insulin level 0-10 min/ fasting glycaemia [20,22] have also been proposed. The main differences between AIR and AUC are related to the fact that AUC is an “absolute” value while AIR takes into account the increment (difference between basal and peak values). Therefore, AIR is probably more precise in approaching the kinetics of insulin secretion. The AIR studies the first phase of insulin secretion, while the area under the curve provides information on the total secretion. Though the area under the curve and the Kg require delayed samples, testing time may be reduced to 10 minutes when only an insulin secretion study is needed. Otherwise, AIR may be calculated replacing insulin by C-peptide measurements, their secretion being equimolar. This possibility is interesting because insulin measurement may be underestimated in peripheric venous blood since 50% of insulin is extracted at the first liver passage. Nevertheless fine kinetic analysis of beta cell function may be difficult to assess because of the long half-life of C-peptide (30 mn) compared to insulin (3 mn), leading to smoothing out of quick variations.

<table>
<thead>
<tr>
<th>FPIR</th>
<th>Sum of serum insulin values at 1 and 3 min or at 2, 3, 5 min or at 1, 3, 5 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>First phase insulin response</td>
<td></td>
</tr>
<tr>
<td>AUC</td>
<td>0-5 min</td>
</tr>
<tr>
<td>Area under the curve</td>
<td>0-10 min</td>
</tr>
<tr>
<td>AIR</td>
<td>mean serum insulin at the different times during 5 or 10 min</td>
</tr>
<tr>
<td>Acute Insulin Response</td>
<td>minus fasting serum insulin (most frequently mean serum insulin at 1, 3, 5 min minus fasting serum insulin)</td>
</tr>
</tbody>
</table>

Table III
Protocol for intravenous arginine test in the Edmonton protocol.

<table>
<thead>
<tr>
<th>Preparation before test</th>
<th>Same as for IVGTT, fasting, on a separate day</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arginine HCl dose</td>
<td>5 g</td>
</tr>
<tr>
<td>Infusion route</td>
<td>Manual, intravenous</td>
</tr>
<tr>
<td>Infusion duration</td>
<td>30 sec</td>
</tr>
<tr>
<td>T0</td>
<td>Start of infusion</td>
</tr>
<tr>
<td>Periodicity of samples</td>
<td>T-10, T0, T+2, T+3, T+4, T+5, T+7, T+10 min</td>
</tr>
<tr>
<td>Catheter</td>
<td>Two</td>
</tr>
<tr>
<td>Calculation of AIRa</td>
<td>Mean of the three highest serum insulin values at 2, 3, 4, and 5 min post infusion minus the mean basal value</td>
</tr>
</tbody>
</table>

Benefits and drawbacks of ivgtt and arginine test

IVGTT

Benefits
The IVGTT is simple and cheap to perform. It is useful to study both insulin secretion and insulin resistance: the AIR studies the first phase of insulin secretion, while the area under the curve provides information on the total secretion, and the Kg (slope of the natural log of the glucose values from 5 to 30) assesses glucose tolerance. Moreover, it offers the possibility to evaluate insulin resistance with the minimal model [27]. Though the area under the curve and the Kg require delayed samples, testing time may be
There are two drawbacks to the test: the high level of hyperglycaemia induced by the glucose bolus is ethically questionable in situations where glucose balance is essential, and intravenous glucose does not reproduce the physiological intake of glucose as it bypasses the gastro-intestinal-linked « incretine-like » effect. The gastro-enteric hormones (mainly GIP and GLP-1) released by enteric cells in reaction to nutrient absorption magnify β cell response to glucose and induce an insulin secretion greater than that reached after intravenous glucose stimulation [28,29,30].

The drawbacks linked to reproducibility have been considered above. Also, all of the considered tests are non physiologic insulin stimuli.

Arginine test

This quick test assesses not only beta cell function with the AIR or AUC, but also counter-regulatory hormones by studying glucagon response to arginine (AGR). Recent studies have shown that insulin resistance is associated with increased AGR and increased suppression of glucagon levels by glucose. Hence, not only the islet beta cells but also the α cells seem to undergo compensatory changes during the development of insulin resistance [31].

The major interest of this test is that it has no influence on glycaemia levels, an especially interesting feature in diabetic patients. Nevertheless rare anaphylactic reactions have been described [32].

GPAIS

The main advantage of this test is to evaluate β and α cell function by estimating the sensitivity of β cells to glucose, the potentiation of glucose to arginine injection, and the inhibition of glucagon secretion by glucose. The main drawback is its difficulty to implement in clinical practice.

Air and islet transplantation

Islet transplantation, proposed in brittle forms of type 1 diabetes or in case of hypoglycaemic unawareness, has become a clinical reality over the five last years [1,2,3,24,33]. Insulin independence is the most important end point, but has to be tempered with acceptable glycaemic control. Indeed islet function can be severely impaired though the subject remains insulin-independent. Suggested tests to monitor islet mass and function include AIRg, AIRa, AUCi (AUC of insulin), insulin response to glucagon and GPAIS [8,34]. Each of these tests should be considered in the light of their specificity, their correlation to islet mass, glycaemic control and future graft survival. In the following part, we would like to highlight the interest of AIR in islet transplantation. Note, however, that it is not necessarily the gold standard, and more elaborate tests providing simultaneous estimates of insulin secretion and action may be preferred.

AIR and islet autotransplantation

Human islet autotransplantations performed between 1992 and 1998 were associated with a decrease of AIRg, AIRa and AIRmax, strongly correlated with the decrease in auto-transplanted islet mass [34,35], as previously demonstrated by Robertson in partial hemipancreatectomy [26] and after segmental pancreas transplantation [36,37]. Robertson’s results in islet autotransplantation showed stable AIRa whereas AIRg tended to decrease over time [38].

Studies performed in animal models provided additional information on the value of AIR at different levels of insulin secretion and showed the lack of insulin resistance even when the AIR had decreased [39]. The site of islet implantation in rats had no influence on the AIR, which was systematically decreased, while graded quantitative infusions of islets appeared correlated to the magnitude of AIR [40,41]. In accordance with these results, we found a positive correlation between AIRg performed one week and one month after islet autotransplantation in minipigs.
AIR and islet allotransplantation

In the first series of 12 transplanted patients with the Edmonton protocol [33], only 19% had normal AIRg and 36% had normal AUCi, even though they had received 85% of a whole pancreas islet mass. This suggests that the surviving islet mass was marginal. The AIRg, AUCi, and AUCc-p (AUC of C-peptide) were correlated with the ischemic index and the number of islets transplanted, despite the fact that there was no obvious relationship between the number of islets and the actual fasting serum glucose or the stimulated glucose level. Nevertheless, the AIRg was not found to be the best indicator of islet mass, in contrast with previous studies of islet auto-transplantation. The AIRg, AUCi and AUCc-p all showed minimal response midtransplant and substantial improvement after the second transplant, but they were still low compared with normal values. There was, however, a significant reduction in exogenous insulin requirements at the midtransplant period. The poor islet response may reflect an initial degranulation of the islet insulin content, or preferential islet destruction after the first transplantation, perhaps because of a lesser degree of immunosuppression.

On a larger series in the same center [24], AIRa correlated better with transplanted islet mass than AIRg or AUCi. AIRg and AUCi were more closely related to glycemic control (especially to fasting glucose and 2-hours OGTT glucose), and cold ischemia index, but the correlation with the number of islets transplanted, which is an approximation, was no longer found. Posttransplant AUCi was lower in patients who eventually became C-peptide deficient [4,24]. Therefore, continued insulin reserve provides long-term glycemic control and, according to these results, the best indicator of insulin reserve appears to be AIRg. In terms of glucose control, it is of interest that the C-peptide response to the mixed meal challenge was normal, albeit with a higher glucose level, yet the response to intravenous glucose was blunted. This might suggest either a loss of islets or poor β cell function [24]. Perhaps the incretin response is more marked in this setting accounting for the better C-peptide response to the oral challenge. Also, AIR tests the first phase of insulin secretion, whereas a mixed meal does not discriminate between the first and the second phase of insulin secretion. But this discrepancy also raises the question of the accuracy of AIR compared to a simpler test such as the mixed meal test. Indeed, this test requires only two blood samples with the drawback of not being appreciated by patients because of the sweet taste, and to possibly greatly increase blood glucose levels.

Besides these markers of islet mass or glucose control, the best predictors of insulin independence are a 50% decline in insulin requirements following the first transplant and lower insulin requirements pretransplant.

Finally, Edmonton results showed that whereas AIRg and AUCi were closely related, there was no significant relationship between AIRg and AIRa in control or study subjects. Thus, although both stimulate insulin release, they are not measuring the same mechanisms. Nevertheless, these data should be confirmed by the experience of other teams.

Specificity of AIR

One of the major roles of the AIR is to evaluate pancreas or islet graft function, independently from the main efficiency criteria, i.e. insulin independence. Insulin independence in islet transplantation was ideally defined as insulin discontinuation with normal fasting and postprandial blood glucose levels, normal glycated hemoglobin, and disappearance of hypoglycemia [45]. The definition evolved towards the idea of partial success when small amounts of insulin were necessary to keep normal glycemic balance. Recently, islet transplantation efficiency was defined according to a clinico-biological score taking into account four parameters: HbA1c, number of insulin units per day or requirement for oral antidiabetic drugs, fasting glycemia and C-peptide stimulation [46].

The course of insulin secretion can be simply and quickly assessed with AIR. Indeed, the choice of the stimulus mainly depends on what you want to study. AIRg and AIRa are correlated in auto-transplanted patients [34], but no correlation could be demonstrated in islet allotransplantation [24,33]. Long-term evaluation of islet function is divergent. The comparison is difficult because of inter-species variability, post-graft timing of evaluation, immunosuppressive regimen and weight loss. While AIRa, and even AIRmax are stable over time in allo- or auto-engrafted patients [35,38,47], AIRg rather seems to decrease with post-graft duration [24,38,39,47] except in a few cases where it increased [48]. AIRg may predict graft survival [24,46] and AIRa may be better correlated with engrafted β cell mass [24]. Last, a correlation between AIRa and the yield of islet isolation has recently been shown in animal models, further suggesting the value of AIRa in assessing beta cell mass [50]. Nevertheless these data have to be confirmed. AIRa offers the advantage of limiting the glucotoxicity of IVGTT. Moreover the quick answer brought by AIRa, together with other predictors of future insulin independence, could be useful to predict the necessity of a second, or even a third islet injection, a potentially risky procedure with about 10% of bleeding. Some examples of AIRa after islet transplantation are given in figure 1.
AIRmax is more difficult to perform than AIRg and AIRa, and does not assess graft function any better. In islet autotransplantation [34,39], AIRmax was strongly correlated with AIRg, and to a lesser extent with AIRa. AIRmax is a reliable assessment of islet mass, and AIRg may be an indicator of insulin supplies. Nevertheless, AIRmax can detect a decrease in insulin supply where classical stimulation tests, especially AIRg, fail [41].

**Conclusion**

While AIR is not commonly resorted to in clinical practice, it is being increasingly used in clinical research. The test is easy, quick and cheap to perform, and offers the advantage of not increasing plasma glucose levels in diabetic patients (with the arginine test). In clinical practice, the calculation of AIR as the mean serum insulin during the five first minutes of the test minus baseline seems to be the most reliable dynamic evaluation of the first phase of insulin secretion.

Divergences between responses to different secretagogues show the complexity of pathophysiological pathways involved. In islet transplantation, AIR is being used to assess the transplanted islet mass while AIRg may predict graft survival [24,50] and AIRa may be better correlated with engrafted β cell mass [24]. These data have however to be confirmed. Even if metabolism studies also require insulin sensitivity analyses, AIR, which evaluates the first phase of insulin secretion, is a useful biological tool with specific indications to assess islet mass, especially in the islet transplantation field. In the future, estimation of islet mass might also be approached with morphological methods (magnetic resonance or positron tomography).

**References**


