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1 Uncovering factors related to pancreatic beta-cell function

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20 **Short title:** Factors related to beta-cell function

21 **Abstract**

22

23 **Aim:** The incidence of type 2 diabetes has increased rapidly on a global scale. Beta-cell
24 dysfunction contributes to the overall pathogenesis of type 2 diabetes. However, factors
25 contributing to beta-cell function are not clear. The aims of this study were (i) to identify
26 factors related to pancreatic beta-cell function and (ii) to perform mechanistic studies *in vitro*.

27 **Methods:** Three specific measures of beta-cell function were assessed for 110 participants
28 who completed an oral glucose tolerance test as part of the Metabolic Challenge Study.
29 Anthropometric and biochemical parameters were assessed as potential modulators of beta-
30 cell function. Subsequent *in vitro* experiments were performed using the BRIN-BD11
31 pancreatic beta-cell line. Validation of findings were performed in a second human cohort.

32 **Results:** Waist-to-hip ratio was the strongest anthropometric modulator of beta-cell function,
33 with beta-coefficients of -0.33 (p=0.001) and -0.30 (p=0.002) for beta-cell
34 function/homeostatic model assessment of insulin resistance (HOMA-IR), and disposition
35 index respectively. Additionally, the resistin-to-adiponectin ratio (RA index) emerged as
36 being strongly associated with beta-cell function, with beta-coefficients of -0.24 (p=0.038)
37 and -0.25 (p=0.028) for beta-cell function/HOMA-IR, and disposition index respectively.
38 Similar results were obtained using a third measure for beta-cell function. *In vitro*
39 experiments revealed that the RA index was a potent regulator of acute insulin secretion
40 where a high RA index (20ng ml⁻¹ resistin, 5nmol l⁻¹ g-adiponectin) significantly decreased
41 insulin secretion whereas a low RA index (10ng ml⁻¹ resistin, 10nmol l⁻¹ g-adiponectin)
42 significantly increased insulin secretion. The RA index was successfully validated in a
43 second human cohort with beta-coefficients of -0.40 (p=0.006) and -0.38 (p=0.008) for beta-
44 cell function/ HOMA-IR, and disposition index respectively.

45 **Conclusions:** Waist-to-hip ratio and RA index were identified as significant modulators of
46 beta-cell function. The ability of the RA index to modulate insulin secretion was confirmed in
47 mechanistic studies. Future work should identify strategies to alter the RA index.

48 **Introduction**

49 The prevalence of type 2 diabetes (T2D) has increased rapidly on an international scale, with
50 pancreatic beta-cell dysfunction and failure at the core of its development [1]. Where
51 hyperglycaemia exists, pancreatic beta-cells must function to a greater capacity in order to
52 produce more insulin to maintain glucose homeostasis [2]. Beta-cells have an ability to
53 functionally adapt to allow for this compensatory response of further insulin production.
54 Beta-cell dysfunction is commonly seen in T2D, where ‘compensation’ of the beta-cells to
55 produce insulin, often due to insulin resistance, leads to the gradual failure of beta-cells [3].
56 With this in mind, there is a need to investigate factors related to pancreatic beta-cell function
57 in humans.

58 Glucose stimulates insulin secretion, triggering and amplifying signals in pancreatic beta-
59 cells [4-6]. Challenge tests such as the oral glucose tolerance test (OGTT) have been used to
60 investigate how effective individuals are at maintaining glucose homeostasis, thus assessing
61 beta-cell function [7]. Progression into T2D status can be categorised by examining
62 alterations in metabolic parameters and beta-cell function. Weir & Bonner-Weir proposed
63 five stages of evolving beta-cell dysfunction during the progression into T2D [8]. Stage 1 is
64 described as ‘*compensation*’, where overweight or obese individuals with a degree of insulin
65 resistance have to increase insulin secretion from beta-cells in order to maintain homeostasis.
66 Stage 2 occurs where fasting blood glucose levels range between 5-7.3mmol/L, which
67 represents ‘*beta-cell adaptation*’. Stage 3 represents ‘*early decompensation*’ in which glucose
68 levels rise above 7.3mmol/L, and from this progress rapidly towards a glucose level

69 representative of stage 4, known as '*stable decompensation*', where levels typically range
70 between 16-20mmol/L. Individuals progressing towards T2D can remain in stage 2 for many
71 years, but when beta-cell mass becomes insufficient at an important point, glucose levels rise
72 rapidly to stage 4. Lastly, stage 5 represents '*severe decompensation*' and extreme beta-cell
73 failure with advancement to ketosis, with blood glucose levels above 22mmol/L. Movement
74 between stages 1– 4 can be in either direction, with diet and exercise interventions having
75 strong potential to return individuals back to stage 2 [8].

76 It is important to identify parameters which influence the function of beta-cells, in order to
77 optimise beta-cell functionality and potentially identify markers of disease progression or
78 targets for intervention. Body mass index (BMI) and an increased energy intake are
79 recognised as major risk factors for conditions associated with beta-cell dysfunction, and
80 although the evidence of a direct effect of BMI on pancreatic beta-cell function is still largely
81 undefined, the association between BMI and T2D has been well established [9-12]. Strong
82 evidence also exists that an excess of visceral fat is closely related to insulin resistance and
83 T2D risk [13]. The above studies did not have beta-cell dysfunction as their primary aim;
84 therefore further research is needed to determine the exact phenotypic and biochemical
85 parameters that influence specific measures of beta-cell function. A number of recent studies
86 have highlighted a link between beta-cell function and high density lipoprotein (HDL)
87 cholesterol [14-16]. Several studies have found links between certain anthropometric and
88 biochemical parameters associated with T2D, with fewer studies examining the determinants
89 of specific measures of beta-cell function in human cohorts. Beta-cell dysfunction is at the
90 core of T2D, therefore it is paramount to understand factors which influence beta-cell
91 function. In contrast to insulin resistance, beta-cell dysfunction continues to be difficult to
92 measure and monitor, due to factors such as inaccessibility to the endocrine pancreas and

93 incretin effects [17]. There is a clear need for the identification of markers that could be
94 assessed in a fasting biological sample, to allow for the assessment of beta-cell function.

95 Therefore, the aim of this study was to investigate and identify potential factors related to
96 beta-cell function measures in a human cohort and to further investigate these *in vitro* where
97 possible.

98 **Material and methods**

99 **Study population**

100 This research focuses on data obtained from the Metabolic Challenge (MECHE) study which
101 is part of a national research program by the Joint Irish Nutrigenomics Organisation, as
102 previously described [18]. The MECHE study recruited 214 healthy participants aged
103 between 18-60 years. Individuals were informed about the purpose of the study and the
104 experimental procedures, prior to giving written consent. Good health was defined as the
105 absence of any known chronic or infectious disease and this was verified by a number of
106 fasting blood tests. Details of the study have been published elsewhere [18-21]. Ethical
107 approval was obtained from the Research Ethics Committee at University College Dublin
108 (LS-08-43-Gibney-Ryan) and the study was performed according to the Declaration of
109 Helsinki.

110 Baseline blood samples were collected on the morning of the study visits following an
111 overnight fast. Participants underwent an OGTT according to the guidelines set by the World
112 Health Organisation/International Diabetes Federation. Venous blood samples were taken
113 before (0 min) and during the OGTT at set time-points (10, 20, 30, 60, 90 and 120 min), and
114 serum and plasma samples were collected as previously described [18-21].

115 Details of the analytes and methods used are previously reported, along with the
116 measurement of cytokines and hormones [19]. Lipidomic analysis was performed on serum
117 samples (BIOCRATES Life Sciences AG, Innsbruck, Austria), and ceramides were measured
118 using an in-house lipid assay as previously described [18].

119 For the present study, participants from the MECHE study who underwent an OGTT and
120 who had valid glucose and insulin data at time-points 0 and 30 min were included (n = 110).
121 Their baseline demographic and biochemical parameters were used for analysis. The
122 validation cohort, (Food for Health (FHI) cohort) comprised of 47 healthy overweight and
123 obese participants, with a mean age of 53 years and a mean BMI of 32.1kg m⁻².

124 **Measurement of beta-cell function and RA index**

125 Beta-cell function was calculated as the ratio of the incremental insulin to glucose response
126 over the first 30 min of the OGTT ($\Delta\text{Insulin}_{30}/\Delta\text{Glucose}_{30}$) and three different measures
127 were employed. Firstly, beta-cell function was adjusted for homeostatic model assessment of
128 insulin resistance (HOMA-IR) ($(\Delta\text{Insulin}_{30}/\Delta\text{Glucose}_{30})/\text{HOMAIR}$). Secondly, the oral
129 disposition index (DI), which takes into account insulin sensitivity, was calculated for all
130 participants ($\Delta\text{Insulin}_{30}/\Delta\text{Glucose}_{30} \times \left(\frac{1}{\text{fastingInsulin}}\right)$ [22]. Thirdly, beta-cell function was
131 calculated and adjusted for the Matsuda Index $10000/\sqrt{(\text{Glucose}_0 \times \text{Insulin}_0 \times$
132 $\text{Glcucose}_{120} \times \text{Insulin}_{120})}$ (, where glucose is in mg dl⁻¹and insulin in iU ml⁻¹[23].
133 Additionally, C-peptide data was substituted for insulin data for the DI, for the beta-cell
134 function ($\Delta\text{Cpeptide}_{30}/\Delta\text{Glucose}_{30}$), and beta-cell function adjusted for the Matsuda
135 Index($\Delta\text{Cpeptide}_{30}/\Delta\text{Glucose}_{30} \times \text{Matsuda Index}$).

136 A ratio of resistin (R₀) to adiponectin (A₀) (RA index) was formulated as follows:

137
$$\text{RA index} = R_0/A_0$$

138 Where: R_0 = fasting plasma resistin levels (ng ml^{-1})

139 A_0 = fasting serum total adiponectin levels ($\mu\text{g ml}^{-1}$)

140 **Cell culture and treatment**

141 All chemicals were purchased from Sigma-Aldrich Ireland unless otherwise stated. Culture
142 media and its related components were purchased from Gibco (Glasgow, UK). The BRIN-
143 BD11 cell line was used in this study [24] and was maintained as previously described [25].

144 For experimental treatments, cells were seeded at a density of 1.5×10^5 cells per well in a 24
145 well plate for insulin secretion assays. Cells were allowed to attach for 24 h before being
146 treated with recombinant rat resistin (Cambridge Biosciences, Cambridge, UK) or rat
147 GACRP30/Adiponectin (Sigma-Aldrich) or ratios of both, for 24 h. Concentrations of 10-
148 20ng ml^{-1} of resistin and 5-20nmol l^{-1} of globular (g) adiponectin were used. Concentrations
149 were chosen in accordance with previous studies [26, 27]. Cells between passage 23-33 were
150 used and all experiments were $n=4$ unless otherwise stated.

151 **Acute insulin secretion**

152 Following the 24 h treatment period, the culture medium was removed and the cells were
153 washed with phosphate buffered saline (PBS). The cells were then incubated with Krebs-
154 Ringer bicarbonate (KRB) buffer (115mM NaCl, 1.28mM CaCl_2 , 4.7mM KCl, 1.2mM
155 KH_2PO_4 , 1.2mM $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 10mM NaHCO_3 , 5 g l^{-1} BSA, all at pH 7.4) supplemented
156 with 1.1mM glucose for 40 min. The media was then replaced with KRB buffer containing
157 16.7mM glucose + 10mM alanine, for 20 min. Following this, the samples were transferred
158 to Eppendorfs and centrifuged, before removing the supernatant and assaying for insulin
159 content using a Mercodia Ultrasensitive Rat Insulin ELISA kit (Mercodia AB, Uppsala,
160 Sweden).

161 **Measurement of mitochondrial membrane potential, intracellular** 162 **calcium and plasma membrane potential**

163 In order to measure mitochondrial membrane potential, a protocol based on Rhodamine
164 fluorescence as described by Wallace *et al* was followed [25]. Cells were treated for 24 h
165 with high and low RA index. Fluorescence was measured over a period of 150 seconds
166 collecting data every 3 seconds, with injection of glucose to a final concentration of 16.7mM
167 + 10mM alanine at 50 seconds.

168 Intracellular calcium was analysed using the FLIPR Calcium 4 assay kit (R8141 Bulk Kit
169 Molecular Devices), as described by Wallace *et al* [25]. Cells were treated for 24 h with high
170 and low RA index. Fluorescence was then measured in a Flexstation, with readings every 2.5
171 seconds for 10 min. Cells were stimulated at 100 seconds with 16.7mM glucose + 10mM
172 alanine).

173 Plasma membrane potential was also determined. Following 24 hour treatment with a high
174 and low RA index, media was removed and the cells were incubated with 100µl of 2.2mM
175 glucose KRB buffer and 100µl of loading dye (FLIPR blue membrane potential buffer
176 (Molecular Devices)) for 20 min. Fluorimetric data was acquired on the Flexstation with an
177 excitation wavelength of 530nm and an emission wavelength of 565nm. The Flexstation was
178 set to run for 350 seconds, collecting data at 2.5 second intervals, with stimulation of the cells
179 (16.7mM glucose + 10mM alanine) occurring at 100 seconds.

180 **Gene expression analysis**

181 Cells were seeded in 6 well plates and allowed to reach 80% confluence before treatment
182 with high RA index and low RA index for 24 h. Total RNA was extracted using TRIzol
183 reagent (Invitrogen). Reverse transcription of 2 µg of total RNA was carried out using

184 random primers and SuperScript II (Invitrogen by Life Technologies). Samples were
185 incubated in a PCR incubator for 25° C for 10 min, 42° C for 50 min and 70° C for 15 min.
186 The expression of *Pancreatic and duodenal homeobox 1 (PDX1)*, *Insulin receptor (INSR)*,
187 *Adiponectin receptor 1 (ADIPOR1)* and *Adiponectin receptor 2 (ADIPOR2)* were
188 investigated by real time PCR on an Applied Biosystems 7900HT fast real-time PCR system
189 using TaqMan gene-specific assays (*PDX1* (assay Rn00755591_m1), *INSR* (assay
190 Rn00690703_m1), *ADIPOR1* (assay Rn01483784_m1) and *ADIPOR2* (assay
191 Rn01463173_m1)). The results were normalised to beta-actin and cyclophilin A expression.

192 **Statistical Analysis**

193 Analysis was carried out using IBM SPSS Statistics V.20. Data are expressed as means ±
194 standard deviation. Linear regression analysis was carried out to examine relationships
195 between beta-cell function and various anthropometric and biochemical parameters.
196 Statistical significance was evaluated using ANOVA with LSD and Bonferroni post-hoc
197 tests. Significant differences were observed if $P \leq 0.05$. For gene expression analysis, primary
198 analysis was carried out using Sequence Detection Software (SDS) 2.4, and secondary
199 analysis used the software package Data Assist 3.01.

200 **Results**

201 **Study population**

202 Analysis was performed on a total of 110 participants who underwent an OGTT. Baseline
203 characteristics are presented in Table 1. An equal gender balance existed with 55 males and
204 55 females. The mean body mass index was 25.3kg m^{-2} , which lies at the lower end of the
205 overweight BMI category ($25.0\text{-}29.9\text{kg m}^{-2}$).

206

207 **Table 1. Baseline characteristics of MECHE cohort (n=110)**

208	Variable	Mean ± S.D.
	Sex (m/f)	55/55
209	Age (y)	32 ± 11
	Weight (kg)	76.65 ± 16.85
210	BMI (kg m⁻²)	25.3 ± 5.3
211	WHR	0.85 ± 0.1
212	BP SYS (mm Hg⁻¹)	123.1 ± 12.9
213	BP DIA (mm Hg⁻¹)	74.7 ± 10.9
214	Glucose (mmol l⁻¹)	5.21 ± 0.56
215	HDL cholesterol (mmol l⁻¹)	1.34 ± 0.36
	TAG (mmol l⁻¹)	1.05 ± 0.60
216	Insulin (µIU ml⁻¹)	8.48 ± 6.69
217	HOMA-IR	2.00 ± 1.70
218	Adiponectin (ug ml⁻¹)	4.99 ± 3.07
219	Resistin (ng ml⁻¹)	4.56 ± 1.77

220 All values are means ± standard deviation. BMI, Body Mass Index; WHR, Waist to Hip Ratio; BP SYS,
 221 Systolic Blood Pressure; BP DIA, Diastolic Blood Pressure; HDL, High Density Lipoprotein cholesterol; TAG,
 222 triglycerides; HOMA-IR, Homeostatic Model Assessment of Insulin Resistance

223 **Identification of factors related to beta-cell function**

224 Gender had no significant relationship with beta-cell function measures. Investigation into the
 225 effect of BMI revealed that as BMI increased, beta-cell function decreased (Table 2). As
 226 described in Table 3 waist-to-hip ratio was the strongest predictor of beta-cell function, with
 227 beta-coefficients of -0.33, -0.30, and -0.26 for beta-cell function/HOMA-IR, DI and beta-cell
 228 function adjusted for Matsuda index respectively. Further examination of the biochemical
 229 parameters revealed that the RA index had a strong relationship with beta-cell function with
 230 beta-coefficients of -0.24 -0.25, and -0.25 for beta-cell function/HOMA-IR, DI and beta-cell
 231 function adjusted for Matsuda index respectively. C12:1(2H) was the strongest predictor of
 232 beta-cell function when ceramide data was examined. The list of ceramides analysed are
 233 present in S1 Table. Additionally, when C-peptide was used to calculate the DI and beta-cell
 234 function adjusted for the Matsuda index similar results emerged (S2 Table).

235

236 **Table 2. Beta-cell function, resistin and adiponectin according to BMI categories**

BMI Categories (kg m⁻²)			
	Group 1 (18-24.9 kg m⁻²)	Group 2 (>25 kg m⁻²)	P
	(n=60)	(n=46)	
Beta-cell function/HOMA-IR (pmol mmol⁻¹)	14.26 ± 10.61	9.55 ± 6.98	0.04
Disposition index (pmol mmol⁻¹)	3.22 ± 2.21	2.48 ± 1.78	0.07
Beta-cell function* Matsuda index	14.50 ± 13.38	10.37 ± 11.94	0.11
Disposition index (C-Peptide) (nmol mmol⁻¹)	3.07 ± 1.97	2.31 ± 2.26	0.07
Beta-cell function (C-peptide)* Matsuda index	24.17 ± 20.06	16.71 ± 16.77	0.05
Resistin (ng ml⁻¹)	4.31 ± 1.54	4.70 ± 1.98	0.25
Adiponectin (µg ml⁻¹)	5.73 ± 3.12	3.55 ± 2.28	<0.001

237 All values are means ± standard deviation. P-value determined using independent samples t-test (Significance
 238 level (P = <0.05)). * indicates multiplication

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253 **Table 3. Linear regression of anthropometric, biochemical and ceramide data against**
254 **beta-cell function measures**

Predictor	Beta-cell function/ HOMA-IR (pmol mmol ⁻¹)		Disposition index (pmol mmol ⁻¹)		Beta-cell function* Matsuda index	
	Beta coefficient	<i>P</i>	Beta coefficient	<i>P</i>	Beta coefficient	<i>P</i>
WHR	-0.33	0.001	-0.30	0.002	-0.26	0.016
RA index	-0.24	0.038	-0.25	0.028	-0.25	0.021
Cer 12:1(2H)	-0.24	0.015	-0.24	0.021	-0.23	0.010

255 Summary of strongest predictors of beta-cell function using linear regression analysis. WHR, waist-to-hip ratio;
256 HDL, high density lipoprotein cholesterol; RA index, resistin-to-adiponectin ratio; cer, ceramide. Data are
257 presented as beta coefficient and P-value according to beta-cell function/HOMA-IR; Homeostatic Model
258 Assessment of Insulin Resistance and DI; Disposition index; beta-cell function (glucose in mg dl⁻¹, insulin in
259 μIU ml⁻¹) adjusted for the Matsuda index; P-value determined using backward linear regression analysis.
260 Significance level = P < 0.05. Demographic and Anthropometric variables included were: age, sex, BMI, WHR,
261 BP SYS, BP DIA. Biochemical variables included were: HDL cholesterol, adiponectin, resistin, RA index,
262 triacylglycerides, Apo E, TNFα, IFNγ, IL2, IL4, IL6, IL8, IL10. Ceramide data from lipidomic analysis was
263 examined. * indicates multiplication.

264

265 To validate the relationship between the RA index and beta-cell function, adiponectin and
266 resistin concentrations were measured in a second cohort. This cohort, the Food for Health
267 (FHI) cohort comprised of 47 healthy overweight and obese, slightly older participants and
268 had a mean age of 53 years and a mean BMI of 32.1kg m⁻². The RA index was successfully
269 validated in this second human cohort with beta coefficients of -0.395 (p=0.006), -0.384

270 (p=0.008) and -0.540 (p<0.0001) for beta-cell function/ HOMA-IR, DI and beta-cell function
271 adjusted for Matsuda index respectively (S3 Table).

272 **RA index modulates insulin secretion in pancreatic beta-cell line**

273 To assess the effects of exposure to resistin, g-adiponectin or a ratio of both, on pancreatic
274 beta-cells, BRIN-BD11 cells were incubated with the adipokines for 24 h. There was no loss
275 of cell viability during the incubation period. Following exposure to resistin no significant
276 effect on insulin secretion was observed (Fig 1a). Conversely exposure to g-adiponectin
277 resulted in a significant increase in insulin secretion at the higher concentration of 20nmol l⁻¹
278 g-adiponectin

279 (Fig 1b). A dose response study of various RA indexes was carried out (S1 Fig). From this
280 dose response data we chose the RA indexes that elicited the lowest and highest insulin
281 secretion response. Following treatment with two different RA indexes, a high RA index
282 (20ng ml⁻¹ resistin, 5nmol l⁻¹ g-adiponectin) significantly decreased insulin secretion whereas
283 a low RA index (10ng ml⁻¹ resistin, 10nmol l⁻¹ g-adiponectin) significantly increased insulin
284 secretion (Fig 1c). Interestingly at this concentration of adiponectin alone there was no
285 significant increase in insulin secretion indicating the importance of the ratio.

286 Functional assays revealed that plasma membrane potential of cells treated with the low RA
287 index was significantly greater in comparison to the control (no treatment). The high RA
288 index displayed significantly lower plasma membrane potential compared to the low RA
289 index (Fig 2). No significant differences were seen between treatments in mitochondrial
290 membrane assays or intracellular calcium assays (S2 and S3 Figs).

291 Real time PCR analysis revealed significant increases in expression of *ADIPOR1* and
292 *ADIPOR2* when cells were treated with low RA index. Treatment with the different RA
293 indexes did not impact on gene expression levels of *PDX1* and *INSR* (Fig 3).

294 **Discussion**

295 The RA index and waist-to-hip ratio were revealed to be strongly associated with pancreatic
296 beta-cell function. The *in vitro* studies support the relationship between the RA index and
297 beta-cell function in terms of insulin secretion. Although previous results have shown the
298 ratio to be a predictor of T2D development, to the best of our knowledge this is the first to
299 report a direct relationship with pancreatic beta-cell function.

300 Dysregulation of adipokine secretion is frequently observed in obesity and T2D [28].
301 Circulating adiponectin in humans typically ranges between 2-30 $\mu\text{g ml}^{-1}$, while the serum
302 concentration of resistin ranges from 7 to 22 ng ml⁻¹ [29, 30]. Adiponectin has been
303 associated with insulin sensitivity and metabolism of lipids in peripheral tissues [31, 32],
304 along with stimulating insulin secretion [33]. Furthermore adiponectin has also been found to
305 exert cytoprotective effects in beta-cells *in vivo*, and aids in protecting cells from undergoing
306 apoptosis [27]. Resistin has been associated with insulin resistance and pro-inflammatory
307 properties, along with impaired insulin secretion, and is believed to be an important link
308 between obesity, insulin resistance and T2D [34, 35]. Resistin treatment impedes glucose
309 tolerance and insulin response in mouse models [36], and modulates cell viability in cell lines
310 [26]. However the translation of these findings to humans has been less conclusive, with
311 mixed findings emerging [37, 38].

312 In support of our results a previous study identified that a resistin to adiponectin ratio was
313 associated with T2D and Metabolic Syndrome (MS) risk [39]. Moreover, this study
314 demonstrated that the ratio of resistin to adiponectin was more strongly correlated with

315 insulin resistance indexes and key metabolic endpoints of T2D and MS than adiponectin and
316 resistin levels alone. This together with our data support the role of the RA index as a
317 potential biomarker of beta-cell function status; use of such a biomarker profile to identify
318 persons at risk of development of T2D could be an important step in the development of
319 targeted lifestyle interventions. Accurate assessment of beta-cell function from a fasting
320 blood sample would allow for earlier identification of beta-cell dysfunction and make it
321 easier to monitor an individual's risk of progression into T2D.

322 Although the present cohort was generally healthy, 46 participants fell into an overweight and
323 obese BMI category ($>25\text{kg m}^{-2}$). Analysis between normal BMI and overweight and obese
324 BMI categories revealed a significant decrease in both beta-cell function/HOMA-IR and DI
325 as BMI increased. Importantly, an intervention study in 11 obese T2D individuals revealed
326 that reducing BMI through energy restriction (600kcal/day) for 8 weeks resulted in
327 significant improvements in beta-cell function [40]. A significant decrease in waist
328 circumference ($107.4 \pm 2.2\text{cm}$ at baseline to $94.2 \pm 2.5\text{cm}$ at week 8) was also observed in the
329 intervention. Based on the present analysis, waist-to-hip ratio was a strong modulator of beta-
330 cell function, when demographic and anthropometric variables were examined. Waist-to-hip
331 ratio emerged as a stronger modulator of beta-cell function than BMI, which is interesting as
332 it therefore may be a better indicator of T2D risk than a BMI score. Supporting evidence for
333 this exists in the literature where waist-to-hip ratio was determined to be a stronger predictor
334 than BMI of T2D risk in a small Taiwanese cohort [41]. This finding also adds to the
335 hypothesis that central obesity and body shape may be important considerations when in
336 assessing T2D risk, due to strong evidence that an excess of visceral fat is closely related to
337 insulin resistance and T2D risk [13]. In a study by Bardini *et al.* (2011), a
338 hypertriglyceridaemic waist phenotype (enlarged waist circumference and increased
339 triglyceride levels) was associated with increased insulin resistance and an overexertion of

340 beta-cell function in participants with normal glucose tolerance, while participants with
341 impaired glucose tolerance and a hypertriglyceridaemic waist phenotype displayed a decrease
342 in beta-cell function. This highlights the importance of implementing an early intervention to
343 decrease T2D risk [42]. Ceramide 12:1(2H) was also predictive of beta-cell function in our
344 cohort. Ceramides are suggested to be responsible for beta-cell apoptosis due to saturated
345 fatty acid exposure, however the mechanism behind how ceramide accumulation leads to this
346 is still unclear [43].

347 *In vitro* verification of the improved beta-cell functionality is an important aspect of this
348 study: the low RA index significantly modulated acute insulin secretion. Functional assays
349 revealed that there was a significant increase in plasma membrane potential in cells treated
350 with the low RA index: this enhancement could underpin the increased insulin secretion
351 under these conditions. Previous studies have examined alteration of plasma membrane
352 potential of cells treated with adiponectin, with mixed findings. A study examining
353 adiponectin treatment in pancreatic islets found no effects on membrane potential, however
354 another study by Wen *et al* investigating adiponectin treatment in hypothalamic cells
355 observed plasma membrane hyperpolarisation [44, 45]. In addition to alterations in the
356 plasma membrane potential significant increases in *ADIPOR1* and *ADIPOR2* expression were
357 observed following treatment with the low RA index. Adiponectin acts by binding and
358 activating *ADIPOR1* and *ADIPOR2*, and the increased expression of both receptors with low
359 RA index treatment suggests that it plays a role in the regulation of beta-cell function [46,
360 47]. This increase in adiponectin receptor expression in conjunction with the alterations in
361 plasma membrane potential provides a potential mechanism for the promotion of insulin
362 secretion under these conditions.

363 Strengths of the present study include directly assessing factors related to specific beta-cell
364 measures obtained during an OGTT and confirmation in an independent cohort. *In vitro*

365 results mirrored the findings in the human studies and provided an opportunity to examine
366 potential mechanisms by which the RA index promoted insulin secretion. Validation of the
367 RA index in the FHI human cohort, a cohort slightly older and with a greater BMI than the
368 MECHE cohort, also strengthens the case of the RA index as a factor related to beta-cell
369 function. The present study population is limited to Irish participants and it is acknowledged
370 that expansion of this research to non-Irish and non-European cohorts would be beneficial in
371 order to fully translate the research findings to the global population.

372 **Conclusions**

373 In conclusion, our findings indicate that waist-to-hip ratio and RA index are strong factors
374 related to pancreatic beta-cell function. Establishing whether alterations in the RA index is a
375 causative factor in development of T2D is a question which remains to be answered.
376 Furthermore, investigation of the ability to modify the RA index through lifestyle
377 interventions will be key to the potential use of such an index. Future work will examine
378 potential mechanisms for modulating the RA index which in turn may lead to new
379 routes/interventions for improving beta-cell function.

380

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383 Catherine Moss and the Conway Institute Core Facility for assistance with PCR experiments
384 and analysis, and also participants of both the MECHE and FHI studies.

385

386

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565

566 **Figure Legends**

567

568 **Fig 1. The effect of 24 hour treatment with resistin, g-adiponectin, or both (representing**
569 **different RA indices) on insulin secretion in BRIN-BD11 cell line.**

570 Values are mean \pm standard deviation (n = 4). *p < 0.05 **p < 0.01 *** p < 0.001. ANOVA
571 was applied across groups with post-hoc LSD test for comparison of resistin, g-adiponectin,
572 and high and low RA index with no treatment (control).

573 **(A)** Cells were incubated for 24 h with 0, 10 and 20ng ml⁻¹ resistin and then stimulated with
574 16.7mM glucose + 10mM alanine to determine insulin secretion.

575 **(B)** Cells were incubated for 24 h with 0, 10 and 20nmol l⁻¹ g-adiponectin, and then
576 stimulated with 16.7mM glucose + 10mM alanine to determine insulin secretion. Overall p-
577 value = 0.00003

578 **(C)** Cells were incubated for 24 h with no treatment (control), high RA index (20ng ml⁻¹
579 resistin, 5nmol l⁻¹ g-adiponectin) and a low RA index (10ng ml⁻¹ resistin, 10nmol l⁻¹ g-
580 adiponectin) and then stimulated with 16.7mM glucose + 10mM alanine to determine insulin
581 secretion. Overall p-value = 0.0003

582

583 **Fig 2. The effect of RA index on the plasma membrane potential.**

584 BRIN-BD11 cells were treated for 24 h with a control (no treatment), high RA index (20ng
585 ml⁻¹ resistin, 5nmol l⁻¹ g-adiponectin) and a low RA index (10ng ml⁻¹ resistin, 10nmol l⁻¹ g-
586 adiponectin). Cells were stimulated with 16.7mM glucose + 10mM alanine at 100 seconds.
587 Data was analysed by determining the difference in relative fluorescence units (RFU)
588 between the average baseline and post stimulation values for each experiment (delta change

589 %). The increase in fluorescence (normalised to baseline) upon stimulation was 26.4% for
590 control, 23.5% for high RA index and 33.9% for low RA index. Statistically significant
591 differences exist upon the increase in RFU between control treatment and low RA index (p=
592 0.009) and high and low RA index (p=0.003). Overall ANOVA $p = 0.007$. Values are
593 represented as mean values (n=5).

594

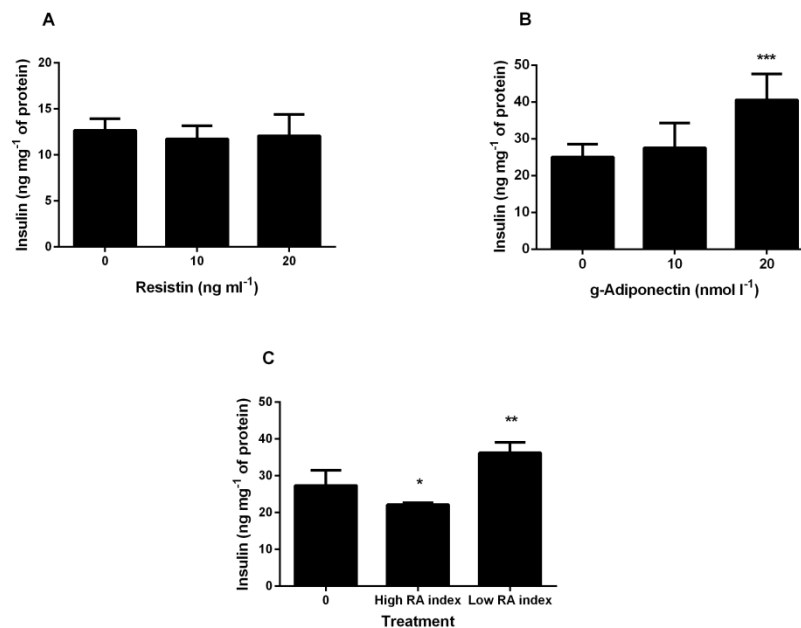
595 **Fig 3. Gene expression analysis of BRIN-BD11 cells treated with RA index.**

596 Low RA index significantly increases (A) *ADIPOR1* and (B) *ADIPOR2* mRNA expression in
597 BRIN-BD11 cells. (C) No effect on *INSR* expression was observed when cells were treated
598 with high and low RA index. (D) *PDX1* expression was not altered by high or low RA index
599 treatment. Experiments n=6, * $p < 0.05$ versus the respective control.

600

601 **Figures**

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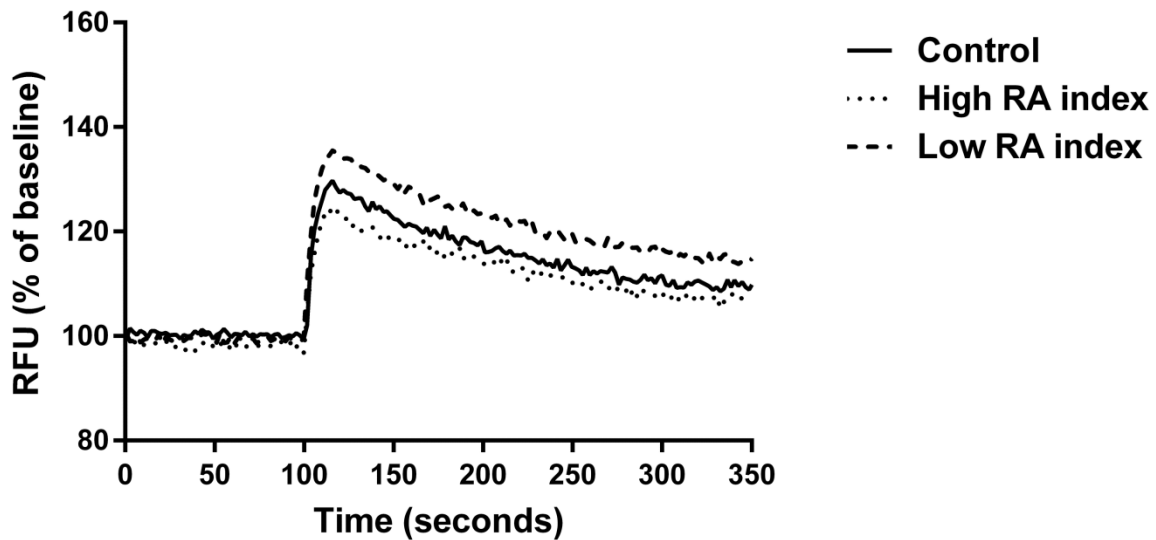
604 **Fig 1. The effect of 24 hour treatment with resistin, g-adiponectin, or both (representing**
605 **different RA indices) on insulin secretion in BRIN-BD11 cell line.**

606 Values are mean \pm standard deviation (n = 4). *p < 0.05 **p < 0.01 *** p < 0.001. ANOVA was
607 applied across groups with post-hoc LSD test for comparison of resistin, g-adiponectin, and high and
608 low RA index with no treatment (control).

609 **(A)** Cells were incubated for 24 h with 0, 10 and 20ng ml⁻¹ resistin and then stimulated with 16.7mM
610 glucose + 10mM alanine to determine insulin secretion.

611 **(B)** Cells were incubated for 24 h with 0, 10 and 20nmol l⁻¹ g-adiponectin, and then stimulated with
612 16.7mM glucose + 10mM alanine to determine insulin secretion. Overall p-value = 0.00003

613 **(C)** Cells were incubated for 24 h with no treatment (control), high RA index (20ng ml⁻¹ resistin,
614 5nmol l⁻¹ g-adiponectin) and a low RA index (10ng ml⁻¹ resistin, 10nmol l⁻¹ g- adiponectin) and then
615 stimulated with 16.7mM glucose + 10mM alanine to determine insulin secretion. Overall p-value =
616 0.0003

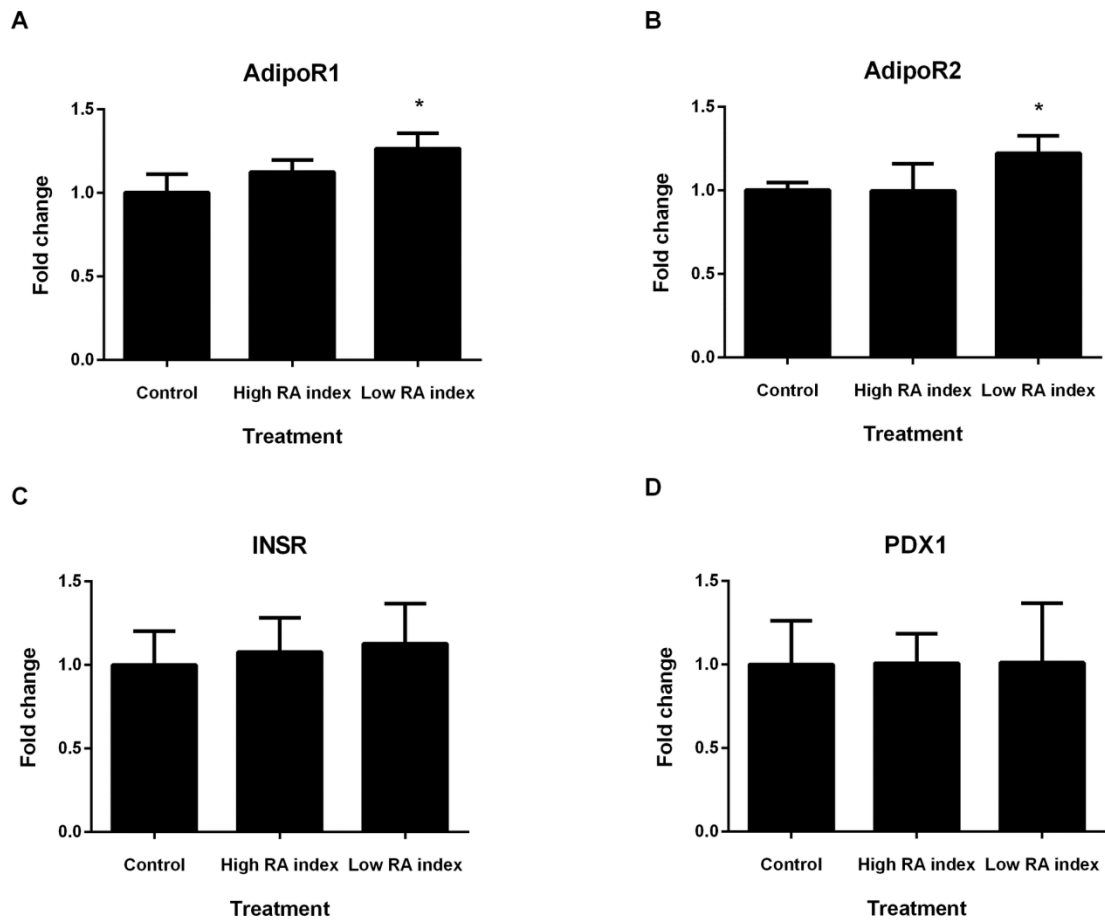


617

618 **Fig 2. The effect of RA index on the plasma membrane potential.**

619 BRIN-BD11 cells were treated for 24 h with a control (no treatment), high RA index (20ng
 620 ml⁻¹ resistin, 5nmol l⁻¹ g-adiponectin) and a low RA index (10ng ml⁻¹ resistin, 10nmol l⁻¹ g-
 621 adiponectin). Cells were stimulated with 16.7mM glucose + 10mM alanine at 100 seconds.
 622 Data was analysed by determining the difference in relative fluorescence units (RFU)
 623 between the average baseline and post stimulation values for each experiment (delta change
 624 %). The increase in fluorescence (normalised to baseline) upon stimulation was 26.4% for
 625 control, 23.5% for high RA index and 33.9% for low RA index. Statistically significant
 626 differences exist upon the increase in RFU between control treatment and low RA index (p=
 627 0.009) and high and low RA index (p=0.003). Overall ANOVA p = 0.007. Values are
 628 represented as mean values (n=5).

629



630

631 **Fig 3. Gene expression analysis of BRIN-BD11 cells treated with RA index.**

632 Low RA index significantly increases (A) *ADIPOR1* and (B) *ADIPOR2* mRNA expression in
 633 BRIN-BD11 cells. (C) No effect on *INSR* expression was observed when cells were treated
 634 with high and low RA index. (D) *PDX1* expression was not altered by high or low RA index
 635 treatment. Experiments n=6, *p < 0.05 versus the respective control.

636

637

638 **Supporting Information**

639 **S1 Table. List of ceramides from MECHE lipidomic dataset.**

CER 7:0(OH)	CER 14:0	CER 19:0(OH)(2H)	CER 24:0(OH)
CER 7:0(OH)(2H)	CER 14:0(2H)	CER 19:0	CER 24:0 (OH)(2H)
CER 7:0	CER 14:1(2H)	CER 19:1	CER 24:0
CER 7:0(2H)	CER 15:0(OH)	CER 19:1(2H)	CER 24:0(2H)
CER 7:1	CER 15:0(OH)(2H)	CER 20:0(OH)	CER 24:1
CER 7:1(2H)	CER 15:0	CER 20:0(OH)(2H)	CER 24:1(2H)
CER 8:0(OH)	CER 15:0(2H)	CER 20:0	CER 25:0(OH)
CER 8:0 (OH)(2H)	CER 15:1(2H)	CER 20:0(2H)	CER 25:0 (OH)(2H)
CER 8:0	CER 16:0(OH)	CER 20:1	CER 25:0
CER 8:0(2H)	CER 16:0(OH)(2H)	CER 20:1(2H)	CER 25:0(2H)
CER 9:0(OH)	CER 16:0	CER 21:0(OH)	CER 25:1
CER 9:1	CER 16:0(2H)	CER 21:0(OH)(2H)	CER 25:1(2H)
CER 10:0 (OH)	CER 16:1	CER 21:0	CER 26:0(OH)
CER 10:0(OH)(2H)	CER 16:1(2H)	CER 21:0(2H)	CER 26:0(OH)(2H)
CER 10:0	CER 17:0(OH)	CER 22:0(OH)	CER 26:0
CER 11:0(OH)	CER 17:0(OH)(2H)	CER 22:0(OH)(2H)	CER 26:0(2H)
CER 11:0(OH)(2H)	CER 17:0	CER 22:0	CER 26:1
CER 11:0	CER 17:0(2H)	CER 22:0(2H)	CER 27:0
CER 11:1	CER 17:1(2H)	CER 22:1	CER 27:1
CER 12: 0(OH)	CER 18:0(OH)	CER 22:1(2H)	CER 28:0(OH)(2H)
CER 12:0	CER 18:0(OH)(2H)	CER 23:0(OH)	CER 28:0
CER 12:1(2H)	CER 18:0	CER 23:0(OH)(2H)	CER 28:1
CER 13:0	CER 18:0(2H)	CER 23:0	
CER 13:0(2H)	CER 18:1	CER 23:0(2H)	
CER 14:0(OH)	CER 18:1(2H)	CER 23:1	
CER 14:0 (OH)(2H)	CER 19:0(OH)	CER 23:1(2H)	

640 CER: ceramide. List of ceramides measured in MECHE serum samples

641

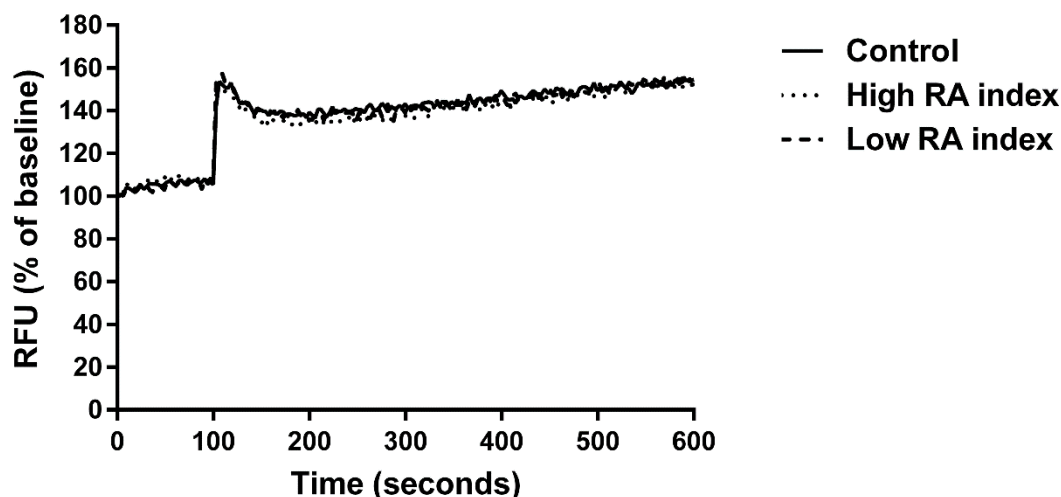
642

643 **S2 Table. Linear regression of anthropometric, biochemical and ceramide data against**
 644 **additional beta-cell function measures**

Predictor	Disposition index (using C-peptide) (nmol mmol⁻¹)		Beta-cell function (C-peptide) *Matsuda index	
	Beta coefficient	P	Beta coefficient	P
WHR	-0.44	<0.001	-0.35	0.001
RA index	-0.28	0.006	-0.20	0.055*
Cer 12:1(2H)	-0.18	0.036	-0.23	0.012

645 Summary of strongest predictors of beta-cell function using linear regression analysis. WHR,
 646 waist-to-hip ratio; HDL, high density lipoprotein cholesterol; RA index, resistin-to-
 647 adiponectin ratio; cer, ceramide. Data are presented as beta coefficient and P-value according
 648 to disposition index, using C-peptide data (nmol mmol⁻¹); beta-cell function using C-peptide
 649 (glucose in mmol l⁻¹, c-peptide in nmol l⁻¹) adjusted for the Matsuda index; P-value
 650 determined using backward linear regression analysis. Significance level = P < 0.05.
 651 Demographic and Anthropometric variables included were: age, sex, BMI, WHR, BP SYS,
 652 BP DIA. Biochemical variables included were: HDL cholesterol, adiponectin, resistin, RA
 653 index, triacylglycerides, Apo E, TNF α , IFN γ , IL2, IL4, IL6, IL8, IL10. Ceramide data from
 654 lipidomic analysis was examined. *RA index in combination with IL-8 was significant
 655 predictor of beta-cell function (C-peptide)* Matsuda index using linear regression (p=0.043).

656



657

658 **S3 Table. Baseline characteristics FHI cohort (n=47).**

Variable	Mean ± S.D.
Sex (m/f)	28/19
Age (y)	53 ± 7
Weight (kg)	94.30 ± 15.35
BMI (kg m⁻²)	32.1 ± 4.6
Waist (cm)	92.69 ± 10.61
BP SYS (mm Hg⁻¹)	127.15 ± 13.69
BP DIA (mm Hg⁻¹)	82.10 ± 8.16
Glucose (mmol l⁻¹)	5.67 ± 0.65
Insulin (μIU ml⁻¹)	12.91 ± 9.79
HOMA IR	3.23 ± 2.41
BCF/HOMA-IR (pmol mmol⁻¹)	11.83 ± 9.03
Disposition index (pmol mmol⁻¹)	2.83 ± 1.96
BCF*Matsuda index	9.45 ± 7.39
Adiponectin (ug ml⁻¹)	11.75 ± 6.44
Resistin (ng ml⁻¹)	9.14 ± 2.97
RA index	0.97 ± 0.51

659 All values are means ± standard deviation. BMI, Body Mass Index; BP SYS, Systolic Blood

660 Pressure; BP DIA, Diastolic Blood Pressure; HOMA-IR, Homeostatic Model Assessment of

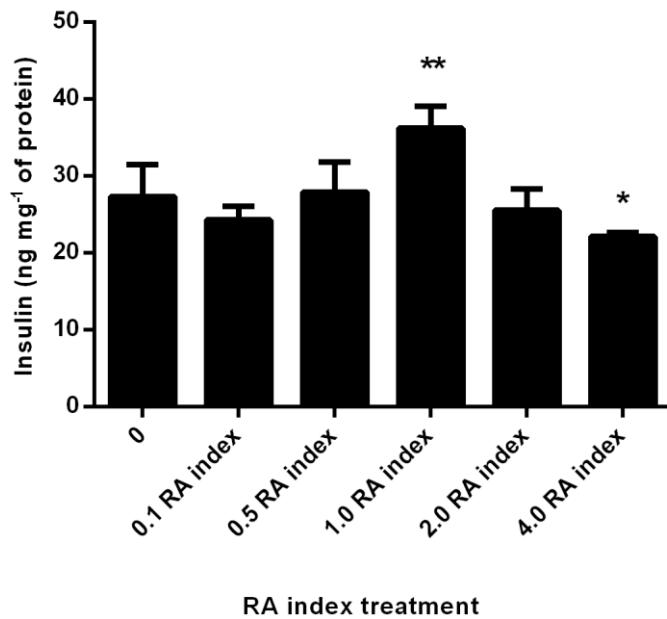
661 Insulin Resistance; BCF/HOMA-IR, beta-cell function adjusted by HOMA-IR;

662 BCF*Matsuda index; beta-cell function adjusted by the Matsuda index (where glucose mg dl⁻¹

663 and insulin μIU ml⁻¹) RA index, resistin to adiponectin ratio

664

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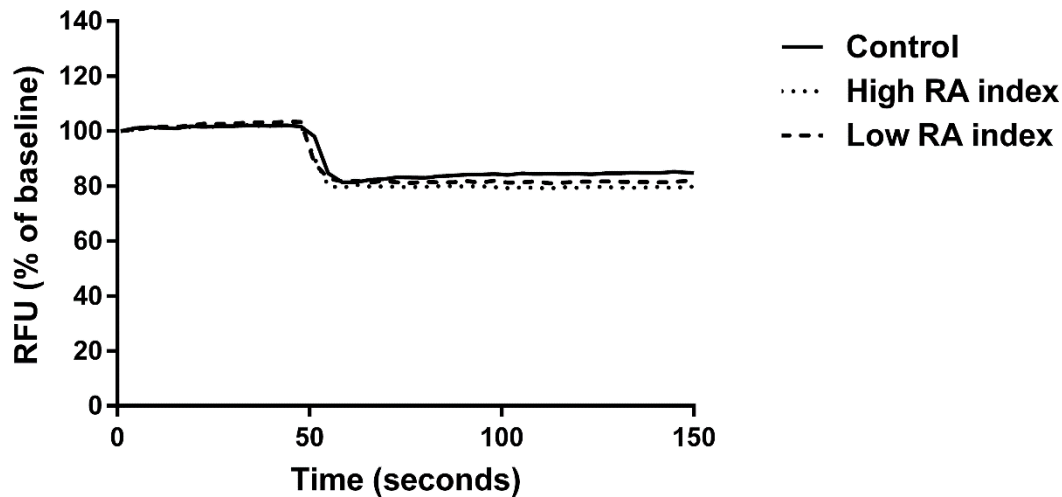
666

667 **S1 Fig. The effect of 24 hour treatment with different RA indices on insulin secretion in**
668 **BRIN-BD11 cell line.**

669 Values are mean \pm standard deviation (n = 4). *p < 0.05 **p < 0.01 *** p < 0.001. ANOVA
670 was applied across groups with post-hoc LSD test for comparison of various RA indexes with
671 no treatment (control).

672 Cells were incubated for 24 hours with no treatment (control), 0.1 ratio (5ng ml⁻¹ resistin and
673 50nmol l⁻¹ g-adiponectin), 0.5 ratio (10ng ml⁻¹ resistin and 20nmol l⁻¹ g-adiponectin), 1.0
674 ratio (10ng ml⁻¹ resistin, 10nmol l⁻¹ g- adiponectin), 2.0 ratio (20ng ml⁻¹ resistin, 10nmol l⁻¹ g-
675 adiponectin) 4.0 ratio (20ng ml⁻¹ resistin, 5nmol l⁻¹ g-adiponectin) and then stimulated with
676 16.7mM glucose + 10mM alanine to determine insulin secretion. Overall p-value = 0.000053

677

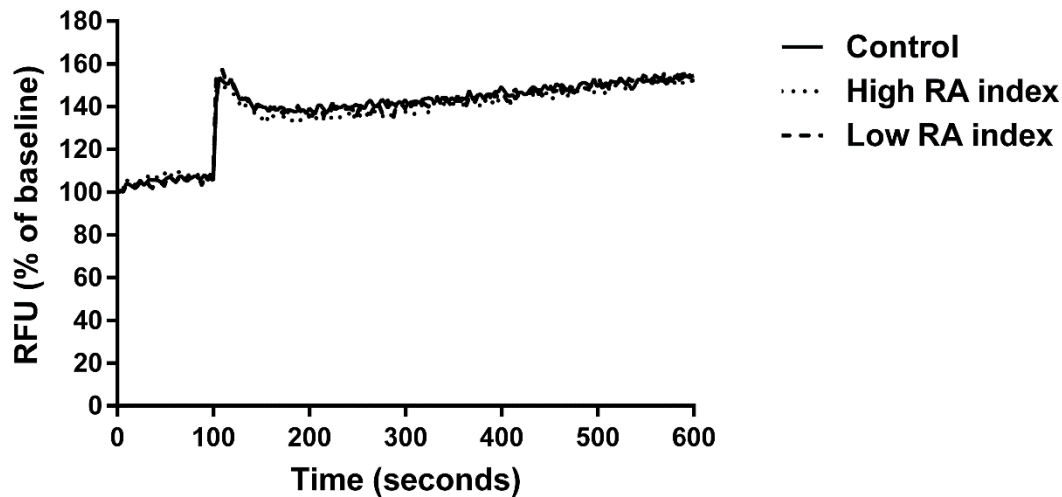


678

679 **S2 Fig. The effect of RA index on changes in mitochondrial membrane potential.**

680 BRIN-BD11 cells were treated for 24 h with a control (no treatment), high RA index (20ng
 681 ml⁻¹ resistin, 5nmol l⁻¹ g-adiponectin) and a low RA index (10ng ml⁻¹ resistin, 10nmol l⁻¹ g-
 682 adiponectin). Cells were stimulated with 16.7mM glucose + 10mM alanine at 50 seconds and
 683 mitochondrial membrane potential was assessed. Data was analysed by determining the
 684 difference in relative fluorescence units (RFU) between the average baseline and post
 685 stimulation values for each experiment (delta change %). The decrease in fluorescence
 686 (normalised to baseline) upon stimulation was 18.9% for control, 21.8% for high RA index
 687 and 20.7% for low RA index. No statistically significant differences exist upon the decrease
 688 in RFU between control treatment and high and low RA index (overall ANOVA p = 0.758).
 689 Values are represented as mean values (n=4).

690



691

692 **S3 Fig. The effect of RA index on changes on intracellular calcium.**

693 BRIN-BD11 cells were treated for 24 h with a control (no treatment), high RA index (20ng
 694 ml⁻¹ resistin, 5nmol l⁻¹ g-adiponectin) and a low RA index (10ng ml⁻¹ resistin, 10nmol l⁻¹ g-
 695 adiponectin). Cells were stimulated with 16.7mM glucose + 10mM alanine at 100 seconds
 696 and intracellular calcium was assessed. Data was analysed by determining the difference in
 697 relative fluorescence units (RFU) between the average baseline and post stimulation values
 698 for each experiment (delta change %). The increase in fluorescence (normalised to baseline)
 699 upon stimulation was 44.3% for control, 40.2% for high RA index and 46.1% for low RA
 700 index. No statistically significant differences exist upon the increase in RFU between control
 701 treatment and high and low RA index (overall ANOVA p = 0.728). Values are represented as
 702 mean values (n=4).

703