Changes of Fatty Acid Composition in Incubated Rat Pancreatic Islets
Evidence for Fatty Acid Release

EF Martins¹, CK Miyasaka², P Newsholme³, R Curi⁴, AR Carpinelli⁴

SUMMARY
Objective: The hypothesis that changes in fatty acid composition of pancreatic islets occur during incubation was investigated.

Methods: The content and composition of fatty acids (FA) from rat pancreatic islets and culture medium after incubation for 1 and 3 hours in the absence or in the presence of 5.6, 8.3, or 16.7 mM glucose were determined by HPLC analysis.

Results: The FA content of pancreatic islets was reduced after 1 hour incubation in the absence of glucose. However, the total FA content was restored by incubating in the presence of 5.6 mM glucose and exceeded by incubating in the presence of 8.3 mM or 16.7 mM glucose. Saturated FA contributed a substantially greater proportion of the total FA increase in comparison to unsaturated FA, being palmitic and stearic acids the most important. The total lipid content of pancreatic islets was not increased if the period of incubation in the presence of glucose was extended to 3 hours. A substantial amount of FA was found in the medium after 1 hour incubation in the absence of glucose: 141 ng per 80 islets for saturated and 75 ng per 80 islets for unsaturated. The release of FA from islets is increased in the presence of glucose.

Conclusion: The release of FA from islets is a novel finding and may be related to modulation of B-cell function.

Key-words: Fatty acid composition - Fatty acid release - Glucose - Pancreatic islets.

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The process of insulin secretion from pancreatic B-cells is stimulated by an increase in glucose metabolism subsequent to an increase in extracellular glucose concentration. An increase in the ATP/ADP ratio results inhibition of K⁺_ATP channels [1, 2], thus depolarising the cell membrane, which results activation of VDCC, an elevation in intracellular Ca²⁺ and stimulation of insulin release [3]. However, several potentiating events involving mitochondrial metabolism and/or activation of protein kinases also occur which modulate insulin secretion [4-6].

FA are important regulators of glucose-induced insulin secretion [7-11]. Acute lowering of plasma FA levels lowers basal insulin secretion [11], whereas increased levels of free FA in fasted mice stimulates in vivo beta-cell electrical activity [12], which is associated with enhanced intracellular calcium concentration and thus insulin secretion. Circulating FA are essential for an efficient glucose induced stimulation of insulin secretion after prolonged fasting in humans [9]. FA have been postulated to activate signalling proteins in pancreatic islets such as protein kinase C, that can potentiate the mechanism of glucose-induced insulin secretion [13, 14].

Intracellular glucose metabolism may additionally promote changes in the phospholipid composition of pancreatic islets [15-17]. Evidence has been accumulated for the synthesis of FA in insulin producing cells [18]. Glucose provides glycerol-phosphate for esterification of FA [9, 19, 20]. Glucose may also be metabolised to citrate in the mitochondria and after being exported to the cytosol may be cleaved to oxaloacetate and acetyl CoA by ATP-citrate lyase. Acetyl CoA carboxylase provides malonyl CoA from acetyl CoA, oxaloacetate and acetyl CoA by ATP-citrate lyase. Acetyl CoA carboxylase provides malonyl CoA from acetyl CoA, oxaloacetate and acetyl CoA by ATP-citrate lyase. Acetyl CoA carboxylase provides malonyl CoA from acetyl CoA, oxaloacetate and acetyl CoA by ATP-citrate lyase.

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Material and methods

Animals

Wistar rats (weighing 200 ± 20 g) obtained from the Department of Physiology and Biophysics, Institute of Biomedical Sciences, USP, were used. The animals were kept in cages in groups of five rats and housed under a light-dark cycle of 12/12 h at 23 ± 2 °C. The rats were fed ad libitum a diet containing 45.5% carbohydrate, 18% protein and 4% lipid and had free access to water. The protocol for utilization of animals was certified on n.174/2001, with the Ethical Principles for Animal Research adopted by the Brazilian College of Animal Experimentation and was approved by the Institute of Biomedical Sciences São Paulo University — Ethical Committee for Animal Research.

Incubation of pancreatic islet

Rat pancreatic islets were isolated as described by Lacy and Kostianovsky [23]. This method is described with details in our previous publications [4, 24, 25]. Batches of 80 islets were incubated in 2 mL of Krebs-Henseleit buffer (in mM: 139 Na⁺, 5 K⁺, 1 Ca²⁺, 1 Mg²⁺, 124 Cl⁻, and 24 HCO₃⁻) at 37 °C for 1 or 3 hours in the absence or in the presence of 5.6, 8.3, and 16.7 mM glucose. After incubation, the medium and islets were removed for analysis.

Lipid extraction and High performance liquid chromatographic analysis of FA

The lipids were obtained from the pancreatic islets and culture medium as previously described [26]. The lipids were saponified using 2 mL of an alkaline methanol solution (1 mol/L NaOH in 90% methanol) at 37 °C, for 2 hours, in a shaking water bath. Afterwards, the alkaline solution was acidified to pH 3 with HCl solution 1 mol/L. FA were then extracted 3 times with 2 mL hexane. After the extraction procedure and saponification [27-29], the FA were derivatized with 4-bromomethyl-7-coumarin [30] and the analysis performed in a liquid chromatograph Shimadzu model LC-10A. The samples were eluted using a C8 column (25 cm × 4.6 id, 5 μm of particles) with pre-column C8 (2.5 cm × 4.6 id, 5 μm of particles), 1 mL per minute of acetonitrile/water (77: 23, by vol) flow and fluorescence detector (325 nm excitation and 395 nm emission) [29]. The FA used as standards were obtained from Sigma Chemical Co. (St. Louis, MO, USA): lauric (C12: 0), myristic (C14: 0), palmitic (C16: 0), palmitoleic (C16: 1-ω6); stearic (C18: 0), oleic (C18: 1-ω9), linoleic (C18: 2-ω6), linolenic (C18: 3-ω6), arachidonic (C20: 4), eicosapentaenoic (C20: 5-ω3) (EPA), docosahexaenoic (C22: 6-ω3) (DHA), and margarine (C17: 0) acids. This latter FA was used to calculate recovery and quantification.

For quantification of FA, we determined the capacity factor (K⁺), elution sequence, linearity, recovery, precision,
Table I
High performance liquid chromatographic analysis of fatty acids composition of freshly obtained and 1 hour-incubated pancreatic islets. The incubation were carried out in the absence and in the presence of different glucose concentrations. The values in ng per 80 islets expressed as mean ± SEM were from four independent experiments using pool of islets from 3 rats each.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>Freshly obtained</th>
<th>none</th>
<th>5.6 mM</th>
<th>8.3 mM</th>
<th>16. mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>12: 0 Lauric acid</td>
<td>82.34 ± 2.13</td>
<td>84.31 ± 6.41</td>
<td>117.65 ± 7.89</td>
<td>111.85 ± 0.88</td>
<td>162.40 ± 23.09a, b</td>
</tr>
<tr>
<td>14: 0 Myristic acid</td>
<td>30.84 ± 1.25</td>
<td>32.16 ± 5.49</td>
<td>54.85 ± 0.81a</td>
<td>54.33 ± 6.93</td>
<td>65.63 ± 3.62a, b</td>
</tr>
<tr>
<td>16: 0 Palmitic acid</td>
<td>247.99 ± 11.48</td>
<td>56.09 ± 15.76a</td>
<td>326.99 ± 14.13a, b</td>
<td>348.83 ± 6.69a, b</td>
<td>378.80 ± 14.78a, b</td>
</tr>
<tr>
<td>16: 1 Palmitoleic acid</td>
<td>2.27 ± 0.20</td>
<td>19.81 ± 0.15a</td>
<td>34.63 ± 2.29a, b</td>
<td>34.61 ± 0.82a, b</td>
<td>24.70 ± 3.82a</td>
</tr>
<tr>
<td>18: 0 Stearic acid</td>
<td>239.64 ± 4.61</td>
<td>65.38 ± 11.77a</td>
<td>346.69 ± 26.41b</td>
<td>402.36 ± 17.48a b c</td>
<td>473.96 ± 10.76a b c</td>
</tr>
<tr>
<td>18: 1 Oleic acid</td>
<td>119.55 ± 5.98</td>
<td>109.19 ± 8.31</td>
<td>157.01 ± 22.94</td>
<td>178.64 ± 7.11</td>
<td>165.94 ± 16.31</td>
</tr>
<tr>
<td>18: 2 Linoleic acid</td>
<td>202.27 ± 0.58</td>
<td>141.45 ± 14.01</td>
<td>174.03 ± 23.13</td>
<td>230.37 ± 18.49</td>
<td>188.24 ± 19.16</td>
</tr>
<tr>
<td>18: 3 γ Linolenic acid</td>
<td>7.94 ± 0.81</td>
<td>9.92 ± 0.89</td>
<td>15.21 ± 1.96</td>
<td>17.91 ± 0.01b</td>
<td>17.35 ± 0.46b</td>
</tr>
<tr>
<td>20: 4 Arachidonic acid</td>
<td>320.31 ± 9.98</td>
<td>120.44 ± 4.64a</td>
<td>149.56 ± 5.33a</td>
<td>124.84 ± 7.02a</td>
<td>136.50 ± 14.32a</td>
</tr>
<tr>
<td>20: 5 EPA</td>
<td>13.35 ± 0.95</td>
<td>9.26 ± 0.38</td>
<td>15.99 ± 2.22</td>
<td>13.01 ± 0.48</td>
<td>13.71 ± 0.19</td>
</tr>
<tr>
<td>22: 6 DHA</td>
<td>22.50 ± 1.63</td>
<td>12.64 ± 0.19a</td>
<td>14.84 ± 1.34a</td>
<td>11.94 ± 1.58a</td>
<td>14.95 ± 0.68</td>
</tr>
</tbody>
</table>

Unsaturation index 2033.18 1045.56 1352.60 1363.81 1323.45

Statistical analysis was performed using ANOVA and Tukey’s test for p < 0.01, where (a) different as compared to freshly obtained islets; (b) different as compared to the condition without glucose (none); (c) different as compared to 5.6 mM glucose. EPA — Eicosapentaenoic acid. DHA — Docosahexaenoic acid. interference, and limit of detection. The minimum limit of quantification of the FA ranged from 1 to 10 pg. We obtained one curve of calibration for each standard, determining coefficients of correlation and regression.

Unsaturation index
The unsaturation index of the FA present in pancreatic islets and culture medium was calculated [31]. The quantity of each FA was multiplied by the number of double bonds present. The total summation was determined and the values are presented in Tables I and II.

Polyunsaturated/saturated (P/S) FA ratio
The P/S ratios of the total FA present in the pancreatic islets and medium were calculated as previously described [32].

Statistical analysis
All results are expressed as the mean and standard error of the mean (SEM), whose applicable. For statistical analysis, ANOVA was used to detect differences among the groups and Tukey’s test was employed to indicate statistical significance at p < 0.01.

Results
Preliminary experiments were performed to determine insulin secretion by the pancreatic islets after 1 hour incubation in the presence of glucose. The values expressed as μU per islet in hour were 60 ± 4 for 5.6 mM, 118 ± 9 for 8.3 mM, and 447 ± 19 for 16.7 mM glucose concentrations (mean ± SEM of 8 determinations). Insulin was determined as previously described [33]. However, the pancreatic islets used in this study are a mixture of various cell types in which B cells represent 60% to 80% of the total. Therefore the changes observed might not reflect exclusively changes in B-cell.

Changes in FA composition of incubated pancreatic islets
The total lipid content of pancreatic islets was substantially reduced after 1 hour incubation in the absence of glucose (Fig 1A). The total lipid content was restored by incubating islets in the presence of 5.6 mM glucose and exceeded by incubating in the presence of 8.3 mM or 16.7 mM glucose. Saturated FA provided a substantially greater proportion of the total lipid in comparison to unsaturated FA (Fig 1A). Palmitic and stearic acids were quantitatively the most important saturated FA increased while linoleic and oleic acids were quantitatively the most important unsaturated FA (Tab I).

The total lipid content of pancreatic islets was not significantly increased if the period of incubation in the presence of glucose was extended to 3 hours compared to 1 hour of incubation (Fig 1B). Likewise the proportion of saturated and unsaturated FA over 3 hours was similar to 1 hour of incubation.
Table II
High performance liquid chromatographic analysis of fatty acid composition of the medium pancreatic islets incubated for 1 hour with different glucose concentrations. The values in ng per 500 µl expressed as mean ± SEM were obtained from four independent experiments using pool of islets from 3 rats each.

<table>
<thead>
<tr>
<th>Fatty acids</th>
<th>None</th>
<th>5.6 mM</th>
<th>8.3 mM</th>
<th>16.7 mM</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0 Lauric acid</td>
<td>56.44 ± 1.00</td>
<td>52.91 ± 2.52</td>
<td>55.17 ± 1.94</td>
<td>54.54 ± 2.53</td>
</tr>
<tr>
<td>14:0 Myristic acid</td>
<td>15.03 ± 0.75</td>
<td>28.98 ± 2.01b</td>
<td>27.01 ± 0.34b</td>
<td>29.61 ± 1.28b</td>
</tr>
<tr>
<td>16:0 Palmitic acid</td>
<td>37.61 ± 2.87</td>
<td>128.60 ± 1.84b</td>
<td>117.74 ± 1.82b</td>
<td>116.48 ± 1.61b</td>
</tr>
<tr>
<td>16:1 Palmitoleic acid</td>
<td>5.91 ± 0.43</td>
<td>10.73 ± 1.68</td>
<td>9.94 ± 0.78</td>
<td>10.97 ± 0.50b</td>
</tr>
<tr>
<td>18:0 Stearic acid</td>
<td>31.84 ± 1.13</td>
<td>172.95 ± 2.13b</td>
<td>156.64 ± 5.13b</td>
<td>156.66 ± 4.46bc</td>
</tr>
<tr>
<td>18:1 Oleic acid</td>
<td>32.65 ± 1.42</td>
<td>44.04 ± 4.87</td>
<td>67.51 ± 0.29bc</td>
<td>67.15 ± 2.50bc</td>
</tr>
<tr>
<td>18:2 Linoleic acid</td>
<td>27.75 ± 1.26</td>
<td>41.46 ± 4.47</td>
<td>79.64 ± 9.02bc</td>
<td>72.11 ± 7.32b</td>
</tr>
<tr>
<td>18:3 γ-Linolenic acid</td>
<td>4.30 ± 0.39</td>
<td>5.66 ± 0.61</td>
<td>9.96 ± 1.04bc</td>
<td>7.72 ± 0.15b</td>
</tr>
<tr>
<td>20:4 Arachidonic acid</td>
<td>&lt; 0.01</td>
<td>0.42 ± 0.01</td>
<td>0.67 ± 0.11</td>
<td>0.60 ± 0.03</td>
</tr>
<tr>
<td>20:5 EPA</td>
<td>4.56 ± 0.13</td>
<td>6.46 ± 0.28</td>
<td>7.71 ± 0.93bc</td>
<td>7.21 ± 0.39b</td>
</tr>
<tr>
<td>22:6 DHA</td>
<td>&lt; 0.01</td>
<td>4.40 ± 0.71</td>
<td>5.48 ± 0.31</td>
<td>6.28 ± 0.54</td>
</tr>
</tbody>
</table>

Unsaturation index | 129.76 | 215.06 | 340.71 | 321.60

Statistical analysis was performed using ANOVA and Tukey’s test for p < 0.01, where (b) different as compared to the condition without glucose (none); (c) different as compared to 5.6 mM group. EPA — Eicosapentaenoic acid. DHA — Docosahexaenoic acid.

Discussion
Pancreatic B cell glucose metabolism results in an increased rate of production of key metabolites, including citrate, which is exported from the mitochondria to the cytosol and acts as a precursor of malonyl CoA synthesis [34]. An increase in malonyl CoA concentration can inhibit carnitine palmitoyl transferase I, leading to a reduction in β-oxidation [35]. There is consequently a rise in concentration...
of acyl-CoA, which has been proposed to modulate insulin secretion [21, 36]. There is evidence that in addition to inhibition of free FA oxidation, glucose-induced insulin secretion is also associated with increased free FA esterification, and complex lipid synthesis by pancreatic B cells [37]. Significant increases have been reported in the total mass of diacylglycerol (DAG) [38], triacylglycerol [39], and phosphatidic acid (PA) [40]. Evidence is presented herein that marked changes of FA composition occur in incubated pancreatic islets. In the absence of glucose FA content decreases, whereas in the presence of glucose, FA content is restored. A significant increase of stearic acid in pancreatic islets was observed after 1 hour incubation in the presence of glucose that remained for up to 3 hours.

In the absence of glucose it is possible that endogenous FA oxidation is increased. As mentioned above, malonyl-CoA produced from glucose metabolism is a potent inhibitor of CPT-1 activity and so FA oxidation [35]. Thus, uninhibited FA oxidation may explain the marked reduction in FA content of pancreatic islets incubated in the absence of glucose. The reduction of polyunsaturated FA was more pronounced than that of saturated FA as indicated by P/S ratio. The precise reason for this observation remains to be clarified. Glucose metabolism therefore preserves the FA content of the islets. Although the mechanism for FA-induced insulin secretion is not fully known, a number of putative mechanisms have been proposed [8]. The role of FA in the pancreatic B-cell includes production of acyl-CoA, phospholipids, DAG, PA, and other signaling metabolites.

Saturated FA with 16 and 18 carbons can markedly enhance glucose stimulated insulin secretion from the perfused pancreas of fasted rats [10]. For example, addition of stearate caused a 21-fold enhancement of insulin release in comparison to 12.5 mM glucose alone. The mechanism for the potent

Figure 1A and 1B
Content of total fatty acids, saturated and unsaturated and P/S ratio in islets freshly obtained or incubated in the absence (none) or presence of 5.6, 8.3 or 16.7 mM glucose for 1 hour (A) and 3 hours (B). The values in ng per 80 islets expressed as mean ± SEM were from 4 independent experiments using pool of islets from 3 rats each. Statistical analysis was performed using ANOVA and Tukey’s test for p < 0.01, where (a) different as compared to freshly obtained islets; (b) different as compared to the condition without glucose (none); (c) different as compared to 5.6 mM group.

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enhancing effect of saturated long-chain FA on glucose stimulated insulin secretion is not known. Evidence is presented that a quantitatively important increase of saturated FA (myristic, palmitic, and stearic) in the incubation medium is observed when islets are incubated in the presence of glucose at all concentrations. FA release from a variety of cell types has previously been described, e.g. adipocytes, cells of the immune system where release is related to cell function (provision of lipid fuel and modulation of the immune response respectively) [41]. The significance of FA release for pancreatic B-cell function remains to be elucidated.

In this study evidence is provided for change in FA composition and content in pancreatic islets incubated in the absence or in the presence of glucose. The changes observed probably reflect changes in content of intracellular triacylglycerol, phospholipid, cholesterol ester, DAG and PA. The process of release of FA from islets reported in this paper is a novel finding and may relate to modulating of B-cell function. Taking into account that a mixture of various cell types is present in pancreatic islets, FA may mediate the interaction between different cells.

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References


Figure 2A and 2B
Content of total fatty acids, saturated and unsaturated and P/S ratio in the medium of islets incubated in the absence (none) or presence of 5.6, 8.3 or 16.7 mM glucose for 1 hour (A) or 3 hours (B). The values in ng per 500 μL of medium expressed as mean ± SEM were from 4 independent experiments using pool of islets from 3 rats each. Statistical analysis was performed using ANOVA and Tukey’s test for \( p < 0.01 \), where (b) different as compared to the condition without glucose (none); (c) different as compared to 5.6 mM group; (d) different as compared to 8.3 mM group.


