SOME CHANGES IN THE FUNCTIONAL AND MORPHOLOGICAL PARAMETERS AFTER VERY LOW CALORIE DIET AND REFEEDING

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ABSTRACT. It was recently found that very low calorie diet (VLCD) and weight reduction have beneficial effect on insulin resistance. AIM: To check how insulin sensitivity changes after VLCD and refeeding. Method: Eighteen male NZW rabbits with similar initial body weight (BW) underwent different diets for 80 days: The VLCD group (n=6) received 30 g chow daily, the CONTR group (n=6) received 150 g chow daily, and the refeeded (REFD) group was treated as VLCD for 80 days and then received 60 g chow daily for a week. At the end of diet period intravenous glucose tolerance test was applied and all the animals were sacrificed under uretan narcosis. Bodies and livers were weighted and cryostat sections for histochemical glycogen determination were prepared from the liver tissue. Results: The serum glucose levels before and after glucose overload did not differ significantly but insulin increased significantly in REFD compared to VLCD (but not to CONTR) at 0, 3rd, 5th, and 60th minutes. Glycogen increases remarkably in hepatocytes of REFD compared to VLCD and seemed more like CONTR’s than like VLCD’s sections. Liver weight and BW of REFD differ significantly with CONTR’s (but not with VLCD’s). Discussions: Our results indicated that the hepatocytes fulfilling and the insulin levels change simultaneously without concordance with body and liver weight changes. We suppose that the insulin serum levels seem associated more with liver glycogen depot fulfilling than with body weight.

KEY WORDS. VLCD, animal, IVGTT, glycogen, liver

INTRODUCTION

In 1991 Per Bjöntorp wrote: „It is increasingly clear that hyperinsulinemia and insulin resistance are obligatory associates with condition that carry an increased risk
of non-insulin-dependent diabetes mellitus. The most common of such conditions is, no doubt, obesity[1]. In humans insulin resistance rises as body mass index increases and adipose tissue is supposed to stimulate insulin resistance via hyperlipidemia and cytokine secretion[2]. Evidences coming from researches on the calorie restriction as one of the most effective methods for weight reduction with highly beneficial effect on insulin effectiveness additionally confirmed the significance of body weight (BW). Very low calorie diet (VLCD) was found to decrease BW [3,4,5], followed by increased insulin sensitivity [6,7]. Most investigations on obesity support the positive correlation between BW and insulin resistance but not in linear fashion. The improvement of glucose metabolism is not always correlated with the degree of weight reduction[5]. For example only 3 days of VLCD normalizes increased glucose levels in obese NIDDM patients and decreases daily insulin needs in insulin-treated patients[8]. Another interesting observation is that modest weight reduction [9,10] decreases insulin resistance, even in obese patients [5]. We suppose that not the body weight is responsible for the changes in insulin effectiveness, but the underlying changes in storage capacity. The abundance of intracellular storages in obesity might decrease insulin effectiveness as a compensatory mechanism against excessive storage accumulation. The beneficial effect of the moderate weight reduction could be explained with a big increase in storage capacity within a small decrease in total body weight. The physiological rationale of storage-dependent insulin sensitivity could be energy redistribution through the body – from less to more loaded depots. In this paper we will report results from an investigation of the relation between hepatocytes glycogen fulfilling and insulin secretion.

MATERIAL AND METHODS
Eighteen male NZW rabbits with similar initial body weight (BW - 2955±245 gr.) and age (6 months old) underwent different diets for 80 days. The VLCD group (n=6) received 30 g chow daily, the control group (CONTR, n=6) received 150 g chow, and the refeeded (REFD) group was treated as VLCD for 80 days and then received 60 g chow daily for a week. At the end of the diet period intravenous glucose tolerance test (IVGTT) was applied in dose of 0.3 g/kg 40% glucose [11] and 0.3 ml blood were withdrawn at minutes -10, -5, 0, 3, 5, 30, 60. Time zero was the start of the infusion. After IVGTT the animals were sacrificed under uretan narcosis. Bodies and livers were weighted and cryostat sections for histochemical glycogen determination (PAS reaction) were prepared from the liver tissue. Guide for the care and use of laboratory animals [12] were followed and our institutional Ethical Committee approved the study protocols. The serum glucose (SG) was measured by glucose oxidase GOD-PAP method using a standard diagnostic kit from Human (Human Gesellschaft für Biochemica und Diagnostica mbH, Germany) and the serum insulin (SI) concentration was assessed by insulin radioimmunoassay (RIA) method with a kit from DiaSorin (DiaSorin s.r.l. Italy). The areas under the curve for insulin (AUCins) were calculated by trapezium method. [13]. Descriptive statistical test and unpaired t-tests were applied. All data
were expressed as means ± SD. Variations were considered statistically significant when p<0.05.

RESULTS

The serum glucose levels before and after glucose challenge did not differ significantly between groups. Serum insulin levels before and during the IVGTT differ significantly between the VLCD and both the REFD and the CONTR but not between the CONTR and the REFD (Table 1). In the first phase of insulin secretion there was a tendency for significant discrimination that could become significant in bigger animal samples. Basal insulin levels and insulin levels in the second phase of insulin secretion were almost identical in the CONTR and the REFD. AUCins in the REFD (818±118) significantly increased compared with the VLCD (500±183; p=0.03) and approached the CONTR values (1531±561; p=0.07).

The CONTR’s BW was significantly higher (4291±144 g.) than both the VLCD’s (3358±156 g; p=0.000001) and the REFD’s (3278±57 g.; p=0.000001) as well as the CONTR’s liver weight (97.25±12.67 g.) compared both to the VLCD’s (58.75±5.97 g; p=0.0002) and to the REFD’s (58.33±2.08 g.; p=0.0006). The VLCD liver and body weights differed not significantly with the ones from the REFD.

The histochemical glycogen determination (PAS reaction) reveal that glycogen in the REFD’s (B) hepatocytes increased remarkably in comparison to the VLCD (C) and seemed more like the CONTR’s (A) than the VLCD’s sections (Figure 1).

DISCUSSION

In this study we did not find association between BW and insulin secretion, both in basal state and after glucose challenge. Quite the contrary: 1) the big differences in BW (between CONTR and REFD) corresponded with similar insulin secretion when hepatocytes glycogen fulfilling is similar and 2) the lack of differences in BW (between the REFD and the VLCD) corresponded with significant increase in insulin secretion when hepatocytes glycogen depot increased. The increment of glycogen contents was followed by increment of serum insulin – both in basal state and after glucose overload. Our data confirmed previous findings that the VLCD decreases liver glycogen[14] but in the literature we did not find any previous demonstration of the positive relation between hepatocytes fulfilling and insulin secretion. We suppose that VLCD causes a total decrement in glycogen storage and the capacity for its restoration: muscle glycogen decreases [15], as well as substrates for gluconeogenesis [16], and hepatic glucose output [9]. It is well known that glycogen itself inhibits glycogen synthase in dose-dependent fashion. Insulin not only stimulates glucose transmembrane influx but also promotes glycogen synthesis via glycogen synthase activation. So decreased sensitivity to insulin action could be, at least in part, due to the changes in glycogen synthase inhibition by intracellular glycogen. In conclusion we suppose that the glycogen storage fulfilling, at least partially, underlie positive correlations between body weight and insulin secretion.
ACKNOWLEDGEMENT
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Table 1. Comparative analysis of insulin (mU/l) dynamics after IVGTT in VLCD, REFD and CONTR groups.

<table>
<thead>
<tr>
<th></th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
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</thead>
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<tr>
<td></td>
<td>0</td>
<td>3</td>
<td>5</td>
<td>30</td>
<td>60</td>
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<td>VLCD</td>
<td>10.24</td>
<td>18.30</td>
<td>18.57</td>
<td>17.68</td>
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<tr>
<td>REFD</td>
<td>22.39</td>
<td>31.33</td>
<td>32.24</td>
<td>29.45</td>
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<tr>
<td>CONTR</td>
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<td>90.46</td>
<td>77.88</td>
<td>28.74</td>
<td>21.44</td>
<td></td>
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|          | 0.0004   | 0.01    | 0.036    | 0.06    | 0.01     |         |
|          | 0.0018   | 0.004   | 0.0007   | 0.02    | 0.004    |         |

p values
VLCD vs. REFD
VLCD vs. CONTR
Some changes in the functional…

Figure 1. Histochemical glycogen determination (PAS reaction) in liver of CONTR (A), REFD (B), and VLCD (C)

REFERENCES


