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Insulin sensitivity in high-producing lactating cows

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Summary

The aim of the studies was to compare lean and obese high-producing milk Holstein-Fresian cows in terms of their insulin sensitivity. 17 animals, 2-8 years of age, weighing 480-725 kg, with a daily milk yield fluctuating between 25.4 and 46.5 kg, were divided into two groups, depending on the body weight and condition: lean and obese cows. Subsequently, both groups were divided into subgroups according to the month of lactation. Their condition was evaluated on a five-point Body Condition Score (BCS) scale. Throughout the entire experiment, the cows were fed ad libitum with the same feed and had free access to fresh water. Blood samples were collected every week directly after milking from the subcutaneous abdominal vein. In the whole blood the glucose level was measured, whereas in the plasma, insulin and FFA concentrations were analysed. On the basis of the results obtained, insulin sensitivity was analysed according to the RQUICKI formula („Revised Quantitative Insulin Sensitivity Check Index"), and the energy balance was calculated. In both experimental groups of lactating cows, the plasma insulin level was found to be several times higher than the physiological norm for non-lactating animals. The highest insulin concentration was noted in obese cows with BCS 3.5. Moreover, in the obese group a high negative correlation (r = –0.62) between insulin sensitivity and BCS, and a positive correlation between the insulin level and BCS were found. The level of free fatty acids (FFA) increased during the lactation, and in both groups it was higher than the reference data. The highest FFA concentration, similarly to insulin, was found in obese cows with BCS 3.5. The glucose level increased during the lactation in both groups of animals, but it was lower than the physiological norm for non-lactating cows. According to our results, there is a relationship between the insulin concentration, insulin sensitivity, and body condition (BCS) in high-producing milk cows. In high-yielding dairy cows elevated levels of FFA may indicate that insulin sensitivity is suppressed. The fat high-producing cows maintained on a positive energy balance are more likely to develop insulin resistance and succumb to production diseases. The RQUICKI test enables an easy assessment of tissue response to insulin.

Keywords: insulin sensitivity, free fatty acids, BCS, lactation, high-producing cows

In recent years it has become clear that a change in insulin sensitivity or a growing insulin resistance (IR) may constitute key etiological factors in the pathogenesis of metabolic or production diseases in cows. IR means that a higher than normal level of insulin (above 5 μU/ml) is required to cope with metabolic demands. The onset of insulin resistance (IR) is connected with the sparing of glucose, increased lipolysis in the adipose tissue and the availability of NEFA for oxidation and milk fat synthesis. In cows, as in humans, any deviation from the norm in insulin action is reflected by fat infiltration, predominantly in the liver, and may be one of the main causes of the fat cow syndrome and reproduction disorders (1-3, 6, 7, 16, 17, 19, 23).

Keeping this in mind, we sought to shed light on changes in insulin sensitivity in high-producing dairy cows in the course of lactation, depending on BCS and fat depot (22, 24, 26). Although the most convenient methods to estimate insulin sensitivity in other species are glucose tolerance tests (GTT), we have chosen the so-called „Revised Quantitative Insulin Sensitivity Check Index" (RQUICKI) since it is more suitable for the analysis of ruminants (7). Earlier studies performed by S. Oikawa et al. (18) focused on the negative energy balance in high-yielding cows as a cause of their impaired health. In our approach, high-yielding cows remained on a positive or neutral energy balance, and we tried to establish why some of these cows were obese while others were lean during the lactation period.

Material and methods

Animals and experimental design. Seventeen Holstein-Fresian cows, 2-8 years of age, weighing 480-725 kg were used in the experiment. Their daily milk yield fluctuated

between 25.4 and 46.5 kg. The animals were divided into two basic groups, lean and obese cows, and then into 5-6 subgroups, according to the month of lactation. All cows were fed *ad libitum* with the same diet, composed of corn silage (46.8%), spent grains (22.27%), TMR (6.38%), hay (4.25%), rape (3.19%), CCM corn (10.63%), and alfalfa silage (6.38%) (30.8-32.35% DM) (9, 10). They also received 300 ml of propylene glycol and had free access to fresh water.

The Body Condition Score (BCS) was determined on a five point scale, based on the amount of fat covering the rump, tailhead and loin area: BCS 1 – deep cavity around the tailhead, no fatty tissue between the pins, the skin is supple and ends of the short ribs are sharp to touch; BCS 2 – shallow cavity around the tailhead, some fatty tissue under the pin bone, ends of the short ribs felt rounded; BCS 3 – no visible cavity around the tailhead, fatty tissue easily felt over the whole rump, ends of the short ribs felt with pressure; BCS 4 – folds of fat around the tailhead, patches of fat visible around the pin bones, the short ribs cannot be felt; BCS 5 – the tailhead buried in fatty tissue, the skin is distended, folds of fat around the short ribs and the whole rump and loin (11). Cows characterized by BCS between 1 and 4 on the five-point scale were used in the experiment.

Blood samples were collected every week, after milking (about 11 a.m.) from the subcutaneous abdominal vein in heparinized test-tubes. In the whole blood the glucose level was measured, whereas in the plasma, insulin and FFA concentrations were analysed.

Hormonal and metabolic parameter analysis. The plasma insulin concentration was measured by radioimmunometric assay (DIA Source, INS-IRMA, Belgium). Free fatty acids were estimated spectrophotometrically according to the Itaya method (8). Forty microliters of the whole blood was mixed with 0.7 ml of 0.9% NaCl and centrifuged at 3000 rpm for 10 min to obtain 0.5 ml of clear supernatant as a test solution. The mixture obtained by this procedure and developed by adding 1.5 ml of a 0.5% solution of a mixture of diphenylcarbazone and diphenylcarbazide (5 : 95) in methanol, was analysed in a spectrophotometer at 550 nm.

RQUICKI (Revised Quantitative Insulin Sensitivity Check Index) analysis was based on plasma concentrations of glucose (Gb) [mg/dL], insulin (Ib) [uU/ml] and free fatty acids (FFAb) [mmol/l] and calculated according to the following formula:

$$RQUICKI = 1/[\log(Gb) + \log(Ib) + \log(FFAb)]$$

A low value indicated decreased insulin sensitivity (7).

The energy balance was calculated using the following formula (13):

$$\mathbf{EB = NE_I - NE_R}$$

where:

$NE_R = (0.9 \times NE_M) + NE_G + NE_L$

$NE_M = (0.359824 \times (0.96 \times BW)^{0.75})$

$NE_L = 0.3886936 \times$ fat % $+ 0.2288648 \times$ crude protein % $+ 0.165268 \times$ lactose % (in milk)

$NE_I = NE_L \times DMI$

$NE_G = (0.035 \times BW^{0.75}) \times (LWG^{1.119}) \times LWG$

DMI – dry matter intake calculated for the diet used

LWG – live-weight gain

The glucose level was estimated using a One Touch II glucometer (Lifescan, Johnson&Johnson Company, USA). The test was performed on fresh full blood immediately after collection.

Results and discussion

The plasma insulin level increased during the lactation period. In both groups and at all sampling times the insulin concentration was higher (9.64-24.29 µU/ml) in comparison with the physiological level in non-lactating cows (0-5 µU/ml). As a result of the relationship between BCS and insulin concentration analysis, the highest level of insulin was found in obese cows with BCS 3.5, whereas the lowest was observed in obese cows with BCS 2.5 (fig. 1). We also estimated the frequency distribution of insulin concentration in all experimental cows. In most lean cows an insulin level of 12-14 µU/ml was observed, whereas in the greater part of obese cows the insulin concentration amounted to 16-20 µU/ml (fig. 2).

The concentration of free fatty acids (FFA) in lean cows was the highest after parturition and decreased during lactation. In obese cows the FFA level was

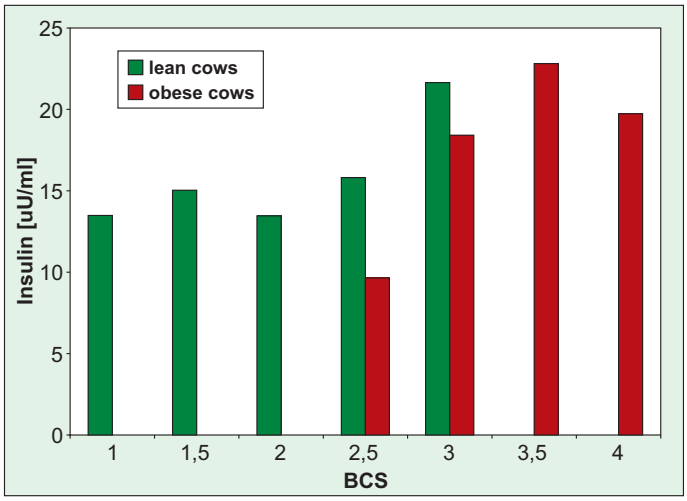

Fig. 1. Relationship between plasma insulin concentration and body condition score (BCS) in lean (n = 9) and obese (n = 8) cows

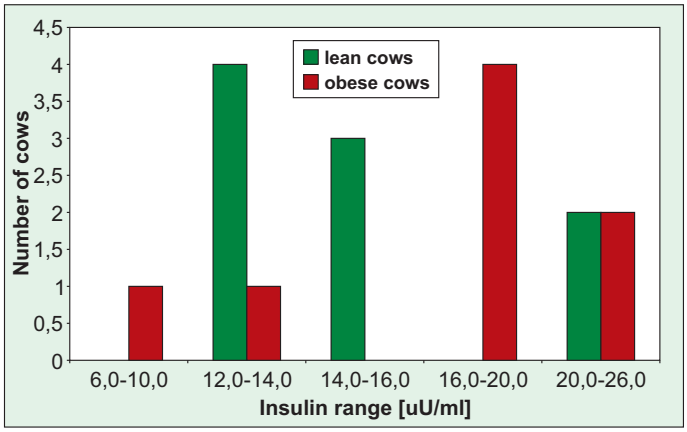

Fig. 2. Frequency distribution of insulin concentration in lean (n = 9) and obese (n = 8) cows

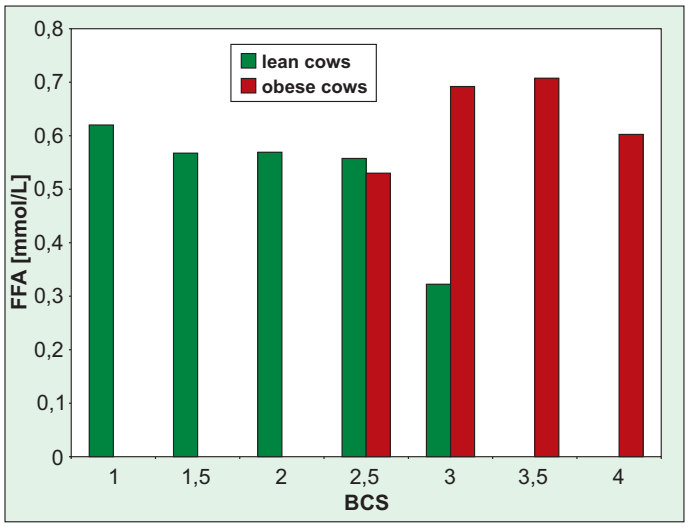

Fig. 3. Relationship between the concentration of free fatty acids (FFA) and body condition score (BCS) in lean (n = 9) and obese (n = 8) cows

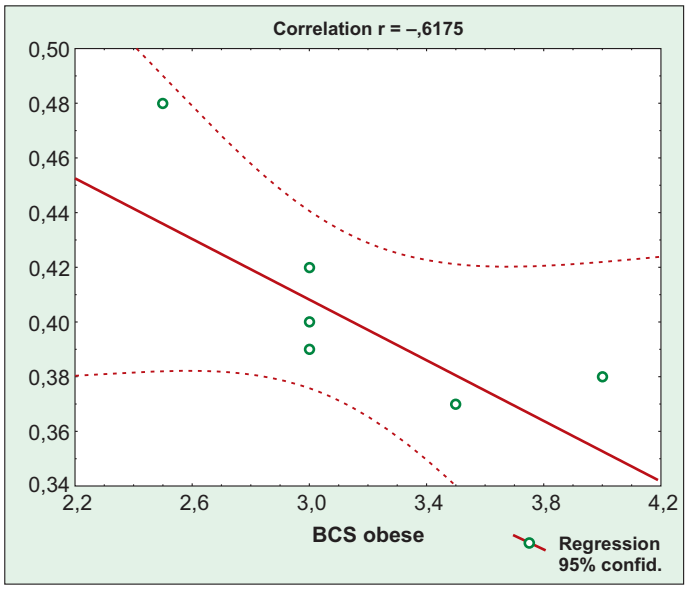

Fig. 4. Correlation between insulin sensitivity (RQUICKI) and body condition score (BCS) (r = –0.62)

always high and reached up to 800 µmol/l in individual samples. In both cases, the FFA concentration was higher than the reference data for non-lactating cows. Free fatty acids were also estimated taking into account the cow's body condition score. The results obtained show that the FFA level fluctuates within a small range in lean cows with BCS from 1 to 2.5, and it is lower than in obese cows with higher BCS. In obese animals the highest FFA serum level was observed in a group of cows with BCS 3.5 (fig. 3).

A high negative correlation (r = –0.6) between BCS and RQUICKI was found in obese cows, which indicates that an increase in BCS leads to a drop in insulin sensitivity (fig. 4).

The serum glucose level was increased during lactation. In lean cows it reached the highest level between the 3rd and the 4th month, whereas in obese cows between the 1st and the 3rd month of lactation. Throughout the experiment, the glucose concentration was lower (0.84-3.52 mmol/L) than the reference data (2.52-4.2 mmol/L) for non-lactating cows (fig. 5).

While glucose tolerance tests (GTT) are the most convenient method of estimating insulin sensitivity in some animal species, they are not suitable for large ruminants. A small dependence of the cellular glucose uptake on insulin (about 20%) (7) and a permanent supply of substrates for gluconeogenesis in the liver marginalize their usefulness in cows. For these reasons we used the so-called Revised Quantitative Insulin Sensitivity Check Index (RQUICKI), well known in human medicine. It is relevant for epidemiological studies of insulin sensitivity and is based on plasma concentrations of glucose, insulin and FFA.

The data concerning insulin resistance (IR) in dairy cows are equivocal, although Oikawa et al. (18) observed decreased tissue responsiveness to insulin with concomitant severe hepatic lipidosis in cows affected

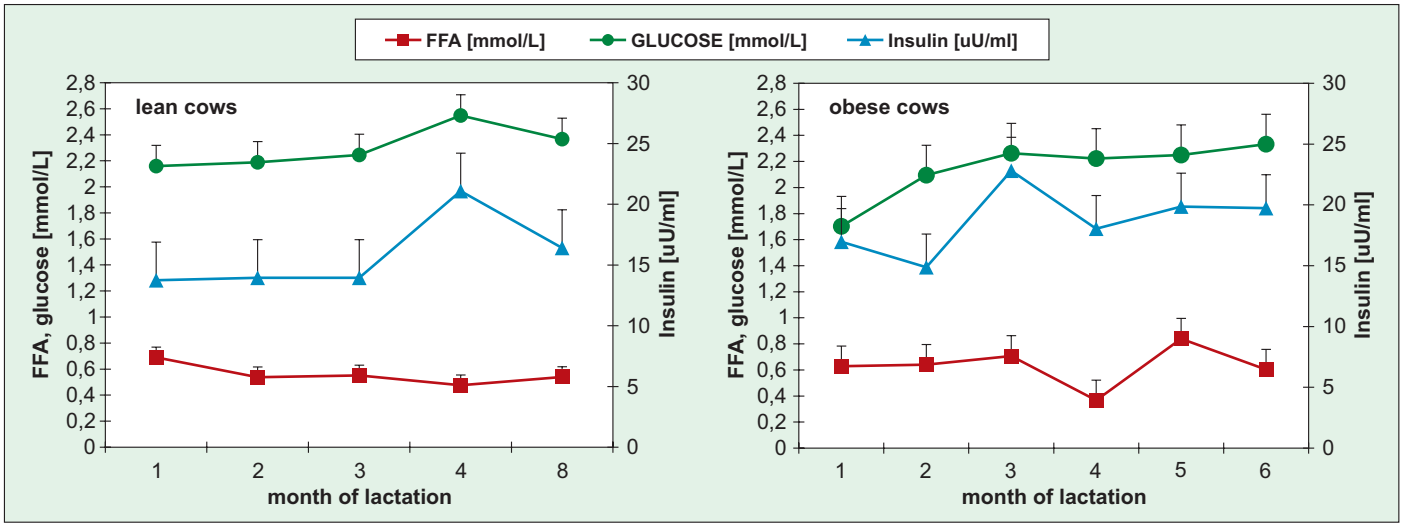

Fig. 5. Blood glucose, plasma free fatty acids (FFA) and insulin concentration throughout the lactation in lean (n = 9) and obese (n = 8) cows. Mean ± standard deviation

by a spontaneous fatty liver. As pointed out by Oikawa et al. (18), the response to insulin in cows treated with TNFα (tumor necrosis factor-α) decreases. It is known that TNFα, as well as other adipokinines, is produced in the adipose tissue. Bearing this in mind, we hypothesised that insulin sensitivity in high-yielding dairy cows may be different, depending on their obesity and body condition scores (BCS).

In the course of lactation, we observed an increase in the insulin level in all experimental cows, but predominantly in the obese cows. In the lean cows the highest level of FFA, observed after parturition, gradually decreased but remained greater than normal (400 µmol/l). In the obese cows the FFA level was permanently high and reached up to 800 µmol/l. In respect to the glucose concentrations, their values successively rose during lactation, although the values were untypically low, even below the reference data (2.5 mmol/l). In nonruminants, both a short- and long-term elevation of plasma FFA concentrations causes IR in the muscle and liver as a result of increases in the intracellular availability of long-chain acyl--coenzyme A and diacylglycerol, which interfere with the intracellular insulin receptor signalling a cascade (21). Our results confirm that in cows, or at least in some of them, insulin elevation is caused by an increase in FFA levels.

Since the above parameters were not relevant for insulin sensitivity, we performed the RQUICKI test. In this case we were also unable to note any significant differences. However, in our cows a very clear inverse relationship was shown between insulin sensitivity estimated by RQUICKI and BCS. Furthermore, cows that scored 3.5 points on the BCS scale had the highest level of insulin (about 25 µU/ml). Additionally, in our experimental dairy cows we found the opposite effects of the BCS index on the level of insulin, which rose, and on the sensitivity to insulin (RQUICKI), which dropped. To sum up, these data seem to suggest that during the lactation period, obesity induces a lower insulin sensitivity in cows and makes them more susceptible to productive disorders such as a fatty liver or infertility (6, 12, 19).

Both lean and obese cows showed an increased plasma level of insulin (three to five times, respectively), but plasma glucose concentrations remained within referential values or even below them (2.2-26 mmol/l). Such an unusual situation cannot be easily explained, but some suggestions can be drawn from the following data. In respect to glucose utilisation by lactating cows there are two contradictory phenomena: in peripheral tissues, such as muscles and adipose tissue, the uptake of glucose declines as a result of increased IR, dubbed as the „diabetogenic effect" (16), but in the mammary gland this uptake systematically grows as a result of increased sensitivity to insulin. The above findings emphasise the considerable role of GH, which on the one hand stimulates the production of FFA but on the other causes a higher level of insulin and a low level of glucose. Since the body mass of these cows ranged from 480 to 725 kg, and the milk yield was above 30 l/day, it may be presumed that they produced a high level of GH, which is known to influence insulin secretion depending on the nutritional status. More recent reports (4) suggest that in dairy cows with a positive energy balance, which was the case in our cows, GH stimulates both insulin release, (by the activation of Janus kinase 2 and mobilisation of intracellular Ca^{2+}) and insulin mRNA expression in beef cows fed to gain weight. Additional support for the suggestion that GH was responsible for such insulin changes comes also from the claim that under its influence circulating FFA concentrations often increase in lactating cows (25). Indeed, in both groups of cows, especially the obese ones, the level of FFA was elevated. Thus, the increased levels of insulin as well as the low levels of glucose observed in our cows could have derived from coordinated metabolic changes induced by GH. Although GH exerts three major effects on carbohydrate metabolism in lactating cows, i.e. increased glucose irreversible loss rate, decreased glucose oxidation, and reduced glucose response to insulin, we are tempted to suggest that the first of these is the most pronounced in high-yielding cows. To expand the role of GH in the observed hormonal and metabolic changes, it is worth mentioning that in fed (as opposed to fasted) ruminants, a high level of glucose does not alter the release of GH (15).

Another reason for a high insulin level in both groups of cows may be their high nutrition status. As it has been reported by some authors (14), plasma insulin increases when cows are on a good feeding regime. In such a case more short and long chain fatty acids are generated, which directly and indirectly stimulates the release of insulin from β cells of the pancreas. However, the elevated level of FFA is responsible for insulin resistance in cows because the reduction of plasma FFA, e.g. by administration of nicotinic acid (antilipolytic agent), enhances glucose clearance (20).

Conclusions

In high-yielding dairy cows elevated levels of FFA may indicate that insulin sensitivity is suppressed. High--producing fat cows maintained on a positive energy balance are more prone to insulin resistance and the development of production diseases. The RQUICKI test is a simple way to evaluate tissue response to insulin.

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