Insulin resistance, renal dysfunction and cardiovascular disease, studies in a high and a low risk population
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CHAPTER 8

General discussion and summary
Introduction

The primary intention for this thesis was to investigate the role of hyperinsulinaemia and insulin resistance in development of renal dysfunction and cardiovascular disease in populations with different degrees of susceptibility. Renal transplant recipients were considered to be highly susceptible compared to the general population. Renal function decline in the general population is commonly stated to be 1 ml/min/year, whereas this is roughly 2-4 ml/min/year in renal transplant recipients. A similar reasoning holds true for cardiovascular disease because renal transplant recipients have been reported to suffer from 3-5 times higher rates of cardiovascular mortality than the general population.

STUDIES IN A HIGH RISK POPULATION: RENAL TRANSPLANT RECIPIENTS

Insulin resistance in renal transplant recipients: validation and determinants

To investigate the role of insulin resistance in epidemiological studies in renal transplant recipients, validated measures of insulin resistance were required. The gold standard to measure insulin resistance is the hyperinsulinemic euglycemic clamp, but this is a cumbersome, difficult and expensive method which is not suited for epidemiological studies. Indices for insulin resistance based on fasting blood parameters alone do not have these disadvantages. However, indices had been validated and established in non-transplant populations, but not yet in renal transplant recipients. In chapter 2 we describe the validation study that we performed in 51 renal transplant recipients in which four surrogate measures of insulin resistance, namely fasting insulin, Homeostasis Model Assessment (HOMA), Quantitative Insulin Sensitivity Check Index (QUICKI), and McAuley’s index were validated against the hyperinsulinemic euglycemic clamp. The Bland-Altman plots indicated that there was sufficient agreement between the gold standard and the four surrogate measures of insulin resistance. This allows the valid use of the indices as measure of insulin resistance in renal transplant recipients longer after transplantation. The best agreement was found for McAuley’s index, likely because the formula included triglyceride concentrations, which are known to be independently correlated with insulin resistance.

The validated indices were used for the study described in chapter 3. In this study, we investigated the determinants of insulin resistance lon-
ger after transplantation, in a larger cohort of 483 non-diabetic transplant recipients. Our results indicate that longer after transplantation obesity and in particular central obesity are the most important determinants of insulin resistance in renal transplant recipients, just like in the general population. This is important as the epidemic of obesity in the general population is paralleled by an epidemic of obesity in renal transplant recipients. Results of studies performed shortly after transplantation suggested that immunosuppressive medication, anti-hypertensive medication, and CMV infection add to insulin resistance after transplantation, but it was unknown whether and to what extend they play a role longer after transplantation. In Chapter 3 it is described that prednisolone dose and tacrolimus use are indeed associated with insulin resistance also longer after transplantation. However, this is not the case for CMV infection longer after transplantation. This may be explained by the fact that shortly after transplantation CMV infections and CMV disease are still accompanied by physical inactivity (caused by malaise in association with the infection) and inflammation, which are likely to resolved longer after transplantation.

**Insulin resistance and muscle mass in renal transplant recipients**

Body composition has important implications in development of disease. Excess fat mass is a well established risk factor for cardiovascular disease (CVD), with insulin resistance is an important underlying mechanism. A less well-known – and underappreciated – fact is that skeletal muscles are an important site of insulin-dependent glucose and fatty acid metabolism. As such, skeletal mass is an important determinant of insulin resistance. Moreover, in insulin resistant states, there may not be only resistance to insulin stimulated uptake of glucose, but there could also be concurrent resistance to insulin stimulation of protein synthesis in muscles. Therefore, the amount of skeletal muscle mass could be related to future morbidity and mortality, and we questioned whether such a relation was dependent on insulin resistance. In this thesis we used 24h urine creatinine excretion as measure of muscle mass.

In the renal transplant population, high muscle mass was associated with low rates of mortality. This association was independent of age, sex, serum creatinine, proteinuria, fasting insulin and insulin resistance related factors (Chapter 4). Consequently, the relation between muscle mass and mortality was independent of the proposed relationship with insulin resistance.
It could well be that low 24h creatinine excretion is just a measure of a general poor health. However, in the statistical models, 24h creatinine excretion predicted adverse events even after adjustment for age (the strongest indicator of poor health), cvd history, amputations and dialysis time. Nevertheless, other (unrecognized or subclinical) co-morbidity for which we did not correct could underlie the results.

A specific co-morbidity that could play a role is sub-clinical protein energy malnutrition. Protein energy malnutrition is a chronic deficiency in macronutrients leading to pathologic depletion of lean body mass, of which muscle mass is the most affected. In the MDRD study, prolonged low protein diets were indeed associated with a decrease in arm muscle area and 24h creatinine excretion.27 In this regard, it is interesting that we found an interaction between creatinine excretion and proteinuria in renal transplant recipients demonstrating that low baseline creatinine excretion is particularly associated with mortality in the presence of proteinuria. This suggests that ongoing proteinuria beyond baseline poses
patients at increased risk for progressive loss of muscle mass and vulnerability for protein energy malnutrition, together resulting in increased risk for mortality. Protein energy malnutrition and tendency for loss of muscle mass could be further aggravated by chronic use of corticosteroids as is common in renal transplant recipients. Another reason why muscle mass is associated with mortality could be related to physical activity. The reasons for this is because in some studies, muscle function is a stronger predictor for all cause mortality than muscle mass.\textsuperscript{28-30} Thus, it seems plausible that greater muscle mass is an indirect measure of physical activity, which could account for our results.

Low muscle mass was also associated with graft failure. It seems unlikely that there is a direct causal relation that lower muscle mass leads to graft failure. Instead a wasting state, inflammation or another (un)known factor more likely predisposes to both loss of muscle mass and decline of renal function decline, leading to graft failure, thereby indirectly associating muscle mass with graft failure. Another possibility is that the muscle wasting is not the cause but the consequence of decreased renal function and its systemic sequela.\textsuperscript{31-34}

STUDIES IN A LOW RISK POPULATION:
GENERAL POPULATION

**Insulin resistance and renal function decline in the general population**

In the general population it might prove to be difficult to demonstrate that hyperinsulinaemia is a factor underlying accelerated decline of renal function. Healthy kidneys seem to be able to recruit otherwise dormant ‘reserve capacity’ in existing nephrons which can replace function of damaged nephrons. Therefore, in a healthy subject endowed with a normal number of nephrons, measured renal function will remain unchanged over a long period of time even with progressive renal damage. This phenomenon is known as single nephron glomerular hyperfiltration, which can mask a possible relation between hyperinsulinaemia and renal function decline. In healthy subjects, recruitment of ‘reserve capacity’ can compensate or even ‘overcompensate’, and thereby mask a decline in renal function. Only after time this initial ‘overcompensating’ hyperfiltration would result in so extensive glomular damage that decline of renal function would become measurable. Recognition of decreased renal function is usually preceded by increased leakage of macromolecules, such as albumin, in the urine. *Figure 1* summarizes a
hypothetical relationship over time of GFR and albuminuria in an insulin resistant compared to an insulin sensitive person. Therefore, one could anticipate effect-modification by age of a relation between fasting insulin and renal function.

In chapter 5 we describe a study in the general population of the PREVEND study in which results were consistent with this scenario. At younger age, those with higher insulin levels had a higher creatinine clearance than those with lower insulin levels. Comparably, at older age those with higher insulin levels had lower creatinine clearance and higher albuminuria, which is consistent with the notion of glomerular hyperfiltration. This cross-sectional observation of the PREVEND study is an indication that insulin resistance could accelerate the age associated decline of renal function. Prospective analyses are required to indicate whether insulin resistance is indeed associated with renal function decline in the general population.

**Insulin resistance, sex and cardiovascular disease in the general population**

It is well known that diabetes mellitus is a stronger CVD risk factor in women than in men.\(^{36-38}\) In women, diabetes increases the risk for CVD was roughly four-fold, but in men diabetes only doubles the risk for CVD.\(^{36-38}\) It was unknown whether this is the consequence of elevated levels of glucose or insulin. It was also unknown whether in non-diabetic persons in the general population, insulin resistance as reflected by hyperinsulinaemia differs between the sexes for an association with CVD risk. We hypothesized that the sex difference would show that women have a higher CVD risk with elevated fasting insulin concentrations compared to men.

Fasting insulin as surrogate marker of insulin resistance has been investigated as a CVD risk factor in more than 30 original studies\(^{39-71}\) and three meta-analyses,\(^{72-74}\) indicating a weak association between fasting insulin and CVD. However, only two studies addressed whether there is a possible sex difference in the association between insulin resistance and cardiovascular disease.\(^{70,71}\) Also these two studies did not use a specific insulin assay (i.e. with little cross-reactivity with pro-insulin) as we did for the study described in chapter 6. This is important because pro-insulin has a stronger association with future CVD than insulin,\(^{39-41}\) which could confound the results of the earlier mentioned studies.\(^{70,71}\)

We, therefore, specifically investigated the relation between fasting insulin and CVD and we hypothesized that insulin resistance, like diabetes,
could have a sex difference with a greater cardiovascular risk in women than in men. Results presented in Chapter 6 indeed indicate that hyperinsulinaemia is a greater and cardiovascular risk factor in women than in men. Fasting insulin was significantly associated with Major Adverse Cardiovascular Events (MACE) in women, which appeared independent of age, smoking, alcohol consumption, and all separate components of the metabolic syndrome, besides measures of obesity (BMI and waist circumference). In men, the association was independent of age, but not after adjustment for smoking, and alcohol consumption, or components of the metabolic syndrome. Thus, in women fasting insulin is associated with a higher risk for development of CVD than in men, which is similar as to the sex differences observed with diabetes.

Like with the sex difference of diabetes, the underlying reasons for the sex difference of insulin resistance are unknown. Importantly, our finding of a sex difference for insulin before the onset of hyperglycaemia suggests that the sex difference does not lie in an increased susceptibility for the effects of hyperglycaemia in men. It also indicates that the difference between men and women for the association of diabetes with CVD is made long before diabetes is established and that it is in fact insulin resistance and not diabetes which neutralizes the ‘advantage’ that women have over men with respect to risk for CVD.

**Insulin resistance and muscle mass in the general population**

In the renal transplant population, muscle mass was associated with mortality independent of insulin resistance. This could be due to numerous reasons including decreased physical activity, increased co-morbidity and/or malnutrition. One would expect these factors to be less common in the ‘healthy’ general population than in renal transplant recipients. Therefore, in the general population, insulin resistance could be a more important mechanism underlying a possible relation between muscle mass, mortality and CVD.

In Chapter 7 it is described that in women of the general population a doubling of creatinine excretion (logarithm base 2) was independently associated a decreased risk for MACE of 56% (P=0.001). In men the association between muscle mass and MACE was not significant (P=0.2). Creatinine excretion was also independently associated with all-cause mortality in women and men.

The relation between muscle mass and MACE was independent of the proposed relationship of insulin resistance in the general population, like
in the transplant cohort described in Chapter 4. Besides the previously mentioned malnutrition and decreased physical exercise, other co morbidity could also play a role such as:

1. An increased inflammatory milieu. Lower muscle mass is associated with higher circulating acute phase proteins. Furthermore, CRP and TNF-α for example can induce muscle wasting in experimental animal models. However, inflammation does not seem to play a role in the general population because, as described in Chapter 7, women with relatively high 24h creatinine excretion had relatively high concentrations of CRP, and, furthermore, results were independent of CRP in both women and men. Also secondary analyses of the data presented in Chapter 4 indicate that results were independent of CRP.

2. Sub clinical hypothyroidism. Overt hypothyroidism, but also sub clinical hypothyroidism are associated with myopathy and muscle mass wasting which can be ameliorated with treatment. Also sub clinical hypothyroidism is associated with CVD, which could thus link muscle mass with CVD.

3. Genetics. Up to 60% of the variation of muscle mass is thought to be genetically determined (excluding sex), which strongly influences muscle mass in later life.

4. In utero development. According to the Barker hypothesis, fetal undernutrition in middle to late gestation can lead to disproportionate fetal growth, which can result in amongst others low muscle mass, but which can also program later coronary heart disease and development of insulin resistance and diabetes.

The last three possible mechanisms were not investigated in both studies, so we are unable to exclude their role on basis of our data.

Some thoughts for the future

The results of this thesis give rise to some thoughts for future research. These can be organised according to two themes, namely (1) insulin resistance and renal function decline and (2) the complex relation between insulin, diabetes, muscle mass and obesity.

Insulin resistance and renal function decline

Prospective studies could be performed to investigate whether insulin resistance is indeed independently associated with renal function decline. One possibility would be in the general population of the PREVEND
study because prospective data about renal function with three repeated measures over a follow-up of almost 10 years will be available.

In renal transplant recipients we just recently collected data on change in renal function in 20 of the 51 patients examined in the validation study described in Chapter 2. Renal function was again measured with the gold standard (125I-iothalamate clearance) in these renal transplant recipients, 2.4 [2.1-3.5] years after the initial measurement of renal function and insulin resistance. Renal function declined with 2.9 [0.0-12.6] ml/min/year (equivalent to 1.9 [0.0-9.5] percent/year). This renal function decline of roughly 2-4 ml/min/year is similar as described in the literature.1-4 Thus, on basis of the renal function decline, these transplant recipients appear representative to other groups.

Figure 2
Relation between insulin resistance and change in GFR, ERPF and FF in 20 renal transplant recipients with a follow up of a median of 2.4 years

Both insulin resistance and renal function were measured by the gold standard (using the hyperinsulinaemic euglycaemic clamp and 125I-iothalamate clearance respectively).
Figure 2 shows that insulin resistance (low M/I value) was associated with more pronounced decline in renal function (percentage change in GFR per year), although this was not significant (P=0.1, possibly due to a lack of power due to low numbers of subjects) and a significant decline in effective renal plasma flow (percentage change in ERPF per year) (P=0.03). There was no change in filtration fraction (FF), but this could be due to the fact that these renal transplant recipients could not further increase filtration fraction, if it was already at a maximum at inclusion of the study.

We are planning to expand the number of transplant recipients with a repeated measure of renal function from 20 to an as large as possible number of the whole group of 51 patients that participated in the baseline measurements of our study. This will increase the power of the study and hopefully allow for multivariate analyses of the data.

The proof of principle that compensatory hyperinsulinaemia due to insulin resistance leads to renal function decline will require a study of ‘reversibility’, i.e. that a reduction of insulin resistance will lead to a slower decline of renal function over time. A randomized clinical trial could be performed to reduce insulin resistance with a measure of renal function as primary outcome. However, specifically reducing insulin resistance will be challenging because non-pharmacological (i.e. exercise, diet, weight loss) and pharmacological (i.e. thiazolidinediones, tapering corticosteroids) approaches not only reduce insulin resistance, but have other effects as well (i.e. increased insulin secretion, reduced blood pressure, reduced pro-inflammatory visceral fat mass and a decreased resting heart rate\(^{90-96}\)). Thus a trial aimed at reducing hyperinsulinaemia will undoubtedly have pleiotropic effects, which could also cause a slower decline in renal function. Therefore, any effects on renal function would not be solely due to the reduction of insulin resistance.

**A complex relation: insulin, diabetes, muscle mass and obesity**

A remarkable cross-sectional observation was made in both populations, which has received little attention in the studies described in Chapters 4 and 7, but which deserves discussion and possibly future research.\(^{26,76}\) In both populations, those with least muscle mass had lowest rates of obesity, lower fasting insulin levels, but relatively more diabetes (Table 1). Comparably, those with the most muscle mass were more often obese, but remarkably had higher fasting insulin concentrations, yet relatively a lower prevalence of diabetes. Several reasons could account why fasting
<table>
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<th>General Population</th>
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<tr>
<td></td>
<td>&lt;1 SD</td>
<td>&gt;1 SD</td>
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<td><strong>Creatinine excretion, mmol/24h</strong> (median, range)</td>
<td>7.5 [3.1-10.1]</td>
<td>17.3 [12.8-28.5]</td>
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<tr>
<td><strong>Men/Women, n / n</strong></td>
<td>51 / 37</td>
<td>48 / 43</td>
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<tr>
<td><strong>Age, yrs</strong></td>
<td>51±14</td>
<td>46±12</td>
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<tr>
<td><strong>Obesity</strong></td>
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<td></td>
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<tr>
<td><strong>BMI, kg/m²</strong></td>
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<td>27.4±4.6</td>
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<td>14 (14)</td>
<td>21 (22)</td>
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<tr>
<td><strong>Waist circumference women, cm</strong></td>
<td>91 [81-105]</td>
<td>97 [84-106]</td>
</tr>
<tr>
<td><strong>Waist circumference men, cm</strong></td>
<td>101 [87-109]</td>
<td>100 [95-107]</td>
</tr>
<tr>
<td><strong>Central obesity</strong>, n (%)**</td>
<td>46 (52)</td>
<td>46 (51)</td>
</tr>
<tr>
<td><strong>Glucose Metabolism</strong></td>
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<td></td>
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<tr>
<td><strong>Diabetes mellitus, n (%)</strong></td>
<td>22 (25)</td>
<td>12 (13)</td>
</tr>
<tr>
<td><strong>Fasting glucose, mmol/l</strong></td>
<td>5.0±1.2</td>
<td>4.7±1.2</td>
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<tr>
<td><strong>HOMA</strong></td>
<td>2.21 [1.67-3.15]</td>
<td>2.31 [1.73-3.68]</td>
</tr>
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</table>

a) Central obesity defined as ≥88 cm in women and ≥102 cm in men.
b) Fasting insulin determined in non-diabetic subjects.
insulin, diabetes, muscle mass and obesity have such a seemingly complex relation.

Low fasting insulin levels suggest insulin sensitivity, certainly with keeping in mind the results presented in Chapter 2. However, low fasting insulin levels could also suggest β-cell dysfunction and not necessarily insulin sensitivity. This could explain why low insulin levels are linked with a greater prevalence of diabetes because fasting insulin does not necessarily differentiate between β-cell dysfunction or insulin resistance. Low insulin concentrations could also be associated with low muscle mass because without sufficient stimulation of insulin, amino acids can not enter myocytes in a sufficient manner, thereby perhaps limiting protein synthesis to replace or build new muscle tissue.97, 98 Vice versa, hyperinsulinaemia could provide an anabolic stimulus for increased muscle mass. In one study with non-diabetic ESRD patients, HOMA was positively associated with protein synthesis and protein breakdown, but negatively with net protein balance.99 Thus insufficient insulin stimulation could play an important role in protein misbalance and skeletal muscle wasting.

Obesity can fit in this complex relation due to several reasons. One reason is that due to hypoinsulinaemia, lipases are not inhibited, thus triglycerides stored in fat cells are hydrolyzed, reducing fat stores. Also, uptake of fatty acids in fat cells is not facilitated due to a lack of α-glycerol phosphate. α-Glycerol phosphate supplies glycerol that combines with fatty acids to form triglycerides to be stored in fat cells.98 Therefore, hypoinsulinaemia could cause increased degradation of fat cells and a decreased uptake of fat in fat cells. The phenotype of muscle (protein) and adipose (fat) wasting is best recognized in patients with type 1 diabetes. Possibly, such a (relative) hypoinsulinaemia could play a role in the general population and possibly even more so in renal transplant recipients because they are more insulin resistant.

A second reason obscuring the relation between muscle mass and obesity could be relative inaccuracy of measurement of obesity. It should be recognized that body mass index is a poor measure of fat mass. Those with a BMI >27 kg/m² could have predominantly an excess of fat mass, but also a large muscle mass with a ‘relative’ healthy fat mass. The measurement of BMI does not differentiate between the two. Vice versa, a person with an apparent “healthy” BMI of 22 kg/m² could have a low lean body mass and excess fat mass and thus actually have an unfavourable body composition.

Our finding that muscle mass independently predicts CVD on the one
hand and that it has a positive association with fasting insulin on the other hand, may explain why fasting insulin is only weakly associated with development of CVD. It could be that low insulin levels (suggesting insulin sensitivity and therefore for a large part negatively associated with CVD), are in fact to a certain extent also positively associated with CVD through a relation with a low muscle mass. The opposite could be true for high insulin levels and high muscle mass. Both effects together may largely obscure an association of insulin with CVD (mortality). Thus, fasting insulin as measure of insulin resistance and its association with increased CVD risk is likely to be confounded by a negative association between fasting insulin and muscle mass.

A good example of the difference between fasting insulin and insulin resistance comes from a Swedish study. Zethelius et al used the hyperinsulinaemic euglycaemic clamp to investigate insulin resistance in 815 men aged 70 years at baseline. During follow-up for 10 years, baseline insulin resistance strongly predicted development of coronary heart disease, whereas fasting insulin did not. This example underlines the weakness of using fasting insulin concentrations as a marker for insulin resistance. Insulin has such diverse roles that a single insulin concentration probably poorly reflects the complex metabolism of insulin. In summary, the relation between insulin, diabetes, muscle mass and obesity as noted from the studies described in chapters 4 and 7 seems complex. Future studies could investigate these relations to better understand the underlying pathophysiology to overcome sarcopenia and maintain sufficient muscle mass in vulnerable patients such as renal transplant recipients.

Such future studies could measure β-cell function and insulin sensitivity prior to transplantation. This could be coupled with measurements of body composition or even muscle and fat biopsies during the transplantation procedure to perform tissue studies. This research would allow a detailed analysis of the possible role of β-cell (dys)function and insulin resistance in muscle wasting and fat accumulation after transplantation. Accurate measurement of β-cell function, insulin resistance and muscle mass are all challenging. However, using the most accurate and sensitive technique’s possible, perhaps new insights can be gained to better understand this complex metabolism.

Finally, in renal transplant recipients one possible therapy option to maintain muscle mass could be by less use of corticosteroids. This has been a topic of much research, but an interesting notion that has not been addressed is corticosteroid sensitivity. Not all persons are equally
sensitive to corticosteroids, similar to the natural varying sensitivity of insulin. However, transplant recipients receive approximately similar amounts of corticosteroids regardless of corticosteroid sensitivity, which is not the case in diabetic patients, because they receive a titrated dose of insulin depending on insulin sensitivity and expected requirement. A potential role for corticosteroid sensitivity could be further investigated together with β-cell dysfunction, insulin resistance and altered body composition (muscle wasting and fat accumulation), with an accurately measured corticosteroid sensitivity. Potentially this could make it possible to titrate corticosteroids to minimize the side effects.

**Conclusion**

In conclusion, we investigated insulin resistance and hyperinsulinaemia in transplant recipients who were considered at ‘high risk’ compared to the ‘low risk’ general population. In fact, renal transplant recipients had roughly a 7 fold higher risk for mortality when we compared all-cause mortality between the renal transplant cohort and the general population of PREVEND (figure 3). A similar life expectancy as the general population is perhaps not feasible in the near future for renal transplant recipients, but there certainly remains potential for improvement.

**Figure 3**

*All cause mortality in renal transplant cohort compared to the general population cohort investigated in this thesis*

- **General Population Cohort**
  - *n=3432*
  - Crude HR: 6.9 [5.2-9.3], *P<0.001*
  - Age and sex adjusted HR: 6.2 [4.6-8.3], *P<0.001*

- **Renal Transplant Cohort**
  - *n=606*

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<th>Number at Risk</th>
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<th>Renal Transplant Cohort</th>
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<tr>
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<td>100 (solid line)</td>
<td>99 (dashed line)</td>
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<td>1</td>
<td>3432</td>
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<tr>
<td>5</td>
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