

SUMMARY AND CONCLUSIONS

This thesis comprises several original studies and a systematic review, aimed at gaining more insight in the surgical stress response and its attenuation with nutritional interventions containing anti-oxidative supplements.

In this chapter the results of this thesis are discussed by describing the main findings, by making conclusions and by giving future perspectives.

The general introduction, **chapter 1**, provides an overview of the current knowledge on the surgical stress response and an overview of nutritional interventions that attenuate the surgical stress response or nutritional interventions that possibly could. The term surgical stress response seems to be a simplification of a complex mechanism of metabolic derangement. In part this metabolic derangement seems beneficial for healing and therefore recovery ¹. In excess, for instance after surgery, it can be detrimental and gives rise to postoperative morbidity and mortality ². Therefore, attempts trying to attenuate the surgical stress response to optimize recovery seem justified.

Novel insights in the surgical stress response place the occurrence of postoperative insulin resistance central with respect to the metabolic derangement after surgery. And, a close interaction between oxidative stress and insulin resistance is postulated. In this thesis, both are subject of investigation to gain more insight and to try to modulate it.

In **chapter 2**, we present a clinical trial on an enteral supplement mixture with anti-oxidants, such as glutamine, ascorbic acid, alpha-tocopherol, beta-carotene, zinc and selenium. In a randomized setting the supplement was given, via jejunostomy from the first postoperative day for almost a week, to major upper gastro-intestinal tract surgery patients. We investigated the effect of the supplement on circulating factors of the anti-oxidant defense system and on markers of oxidative stress.

A main finding of this study was that the blood levels of anti-oxidants decreased significantly after major surgery, as shown by decreased levels of glutamine, ascorbic acid, alpha-tocopherol, beta-carotene, zinc and selenium on the first postoperative day ³. Despite supplementation, only selenium and glutamine rose to preoperative levels after a week ³.

Another main finding was that supplementing anti-oxidants decreased the blood level of lipopolysaccharide binding protein, an acute phase protein that modulates the immune response to endotoxins, by binding to endotoxins and presenting this complex to immune cells ^{3,6}. This finding could suggest that the supplemented anti-oxidants might have protected the integrity of the gut against oxidative stress damage ^{3,6}.

Unfortunately, we were unable to detect surgery induced oxidative stress, by measuring urine F2-Isoprostane, serum malondialdehyde and blood glutathione peroxidase. Additionally, we could not find an effect of the supplemented anti-oxidants on these oxidative stress markers.

The effect on circulating levels of immune-inflammatory markers of this study in major upper gastro-intestinal surgical patients is described in **chapter 3**. As expected, a profound immune-inflammatory response was observed in the supplemented as well as in the control group. The main finding of this part of the study is that the results are indicative that the anti-oxidative supplement attenuated the inflammatory response, by showing lower C-reactive protein levels during the total study period in the supplemented group and by showing a stabilization of the soluble tumor necrosis factor receptor, TNF-R75, in the supplemented group, compared to continue rising in the control group during the study period ⁷. Tumor necrosis factor (TNF) is a pro-inflammatory cytokine, that is inactivated by soluble TNF receptors, like TNF-R75 ⁸⁻¹¹.

International research data point to the importance of starting nutritional interventions in the preoperative setting in order to alter postoperative outcome ¹²⁻¹⁴. Therefore, our next trials incorporated this preoperative start of nutritional intervention. In **chapter 4**, we present a randomized, placebo controlled, clinical trial on a preoperative novel drink containing carbohydrates, glutamine and anti-oxidants, such as green tea extract, ascorbic acid, alpa-tocopherol, bêta-carotene, zinc and selenium. The supplement was given three times preoperatively to rectal cancer surgery patients. The effect on postoperative insulin resistance was investigated by measuring insulin sensitivity in the week prior to surgery and on the first postoperative day. No previous study has reported on insulin sensitivity of liver, muscle and adipose tissue simultaneously in subjects with rectal cancer. Therefore, our main finding was the occurrence of extensive postoperative insulin resistance for glucose metabolism, at the peripheral and the hepatic level, as well as postoperative insulin resistance for fatty acid metabolism. At only one of the three levels an effect of the supplement was seen on postoperative insulin resistance. The supplement was able to attenuate, but not prevent, the postoperative peripheral insulin resistance compared to the placebo drink.

The supplement was originally designed to modulate the inflammatory and oxidative stress response after surgery, for which we made additional measurements. Another finding, therefore, was a slight indication in attenuating the postoperative inflammatory response, by lower CRP levels in the supplemented group compared to the control group during the study period of a week. Also, we were able to detect a state of increased oxidative stress postoperatively, identified by higher postoperative malondialdehyde levels in both groups. Unfortunately, no effect of the supplement was seen on markers of oxidative stress and antioxidant capacity.

Besides our interest in enteral mixtures of anti-oxidants, our research group has been interested in single nutrient supplementation as well. A renewed focus on taurine of our group led to new research ideas. In **chapter 5** an exploratory study on human taurine metabolism is presented. In patients undergoing hepatic surgery, just after gaining access to the abdominal cavity, blood was drawn and flow measurements were done of various veins in the abdominal cavity. This exploratory study is important, since we revealed for the first time human data on net organ fluxes and fractional extraction rates of three organs, like the gut, liver and kidneys. Another main finding was to show that the gut releases taurine. We concluded that in the fasted state, during surgery, this most likely would be due to uptake of deconjugated taurine containing bile acids¹⁵. Our conclusion is supported by findings in a rat study, where comparable results led to a similar statement since the gut seem to lack appropriate enzymes to produce taurine¹⁶. Before starting a clinical trial with taurine supplementation, a systematic review on the study population of interest, the elderly surgical patient, was performed. In **chapter 6** the value of used preoperative nutritional parameters to predict postoperative outcome, in elderly patients undergoing general surgery, is described. With a systematic approach it was revealed that only preoperative weight loss and preoperative serum albumin are adequate parameters to predict postoperative outcome in the elderly general surgery patients¹⁷.

In **chapter 7**, a randomized, placebo controlled, clinical trial with enteral taurine supplementation is presented. In elderly hip fracture patients undergoing surgery, taurine supplementation was started preoperatively and given up to the sixth postoperative day. The effect of taurine supplementation on postoperative oxidative stress, during a study period of a week, and on postoperative outcome, during a study period of a year, was investigated. The main finding of this clinical trial was that taurine supplementation lowered the postoperative oxidative stress, as shown by lower urinary 8-hydroxy-2-deoxyguanosine levels, blunted plasma malondialdehyde response and a trend towards lower plasma lactate to pyruvate ratio¹⁸.

GENERAL CONCLUSIONS AND FUTURE PERSPECTIVES

The clinical trials presented in this thesis enhance the current knowledge on the surgical stress response. The intense way of measuring the postoperative insulin resistance at three levels at once, as we did in **chapter 4**, was not done before. We showed that major surgery, like rectal cancer surgery, induces profound insulin resistance for glucose metabolism, at the peripheral and the hepatic level, and for fatty acid metabolism.

In the three clinical trials with anti-oxidative supplements it was difficult to show surgery induced oxidative stress. We did show surgery induced oxidative stress in two of

the three trials, as shown in **chapter 4** and **7**, but not in **chapter 2** and **3**. We used various measurements to spot oxidative stress, like markers of lipid peroxidation, such as malondialdehyde, F2-Isoprostane (urine and plasma) and oxidized low-density lipoprotein, a marker of DNA damage, such as urinary 8-hydroxy-2-deoxyguanosine, and an indirect measurement of mitochondrial function, such as the lactate to pyruvate ratio. All known as liable markers. However, even more liable markers of oxidative stress exists for blood and urine, and looking at tissues it becomes even more extensive, which indicates a broad range to spot oxidative stress. Choosing one or even a couple measurements over another can easily exclude the ones that are actually affected in the population under investigation. This same conclusion can be made about markers of the immune-inflammatory response. Besides the numerous markers to choose from, mostly they are measured coming from the circulating compartment, whereas the real action is usually in tissues and organs. Therefore, most of the time we measure indirectly what is actually happening. More focus in future research should be on finding adequate direct markers in tissues and organs or making it more feasible to use these markers. In a review, more than a decade ago, a similar suggestion was made to explore the intracellular and intercellular communication to better understand the potential pivotal role of glutathione as the protector against surgery induced oxidative stress¹⁹. Also, some trials presented data on oxidative stress in other compartments than the circulating one. For instance, in a rat study surgery induced oxidative stress was found in serum and liver tissue²⁰. And, in patients after burn injury, that is known to be accompanied by a sufficient amount of oxidative stress, the anti-oxidative status was measured in blood and skin tissue²¹.

The nutritional interventions with anti-oxidative supplements in the trials of this thesis were to some extent able to attenuate the surgical stress response, but not as vigorous as intended. To some extent we were able to attenuate the postoperative inflammatory response and postoperative oxidative stress, but we were not able to alter clinical outcome. Fortunately we did not harm our patients. Recently published large clinical trials, the REDOXS and the Meta Plus trial, in critically ill ICU patients that received anti-oxidative supplements, similar in content to our trial in **chapter 4**, revealed potential harm with anti-oxidative supplements by indications of increased mortality²²⁻²⁴. As a fact, due to their physical property, antioxidants can become pro-oxidants^{25,26}. And, little is known about the interaction between antioxidants. The hypothesis to attenuate the surgical stress response via reduction of oxidative stress and postoperative insulin resistance with anti-oxidative supplements is still shared internationally. However, it is also still a great effort to gain more insight in the mechanism and to find proper supplements. In my opinion, a translational research approach is best suited for this. Therefore, future studies should focus on exploring the mechanism via measurements

on tissue and organ levels. In addition, intervention studies with antioxidants should be by first exploring the anti-oxidant effect on the found mechanism in tissues and/or organs, before clinical outcome studies. Also, slowly building an adequate mixture by adding an anti-oxidant per anti-oxidant in time will give more insight in anti-oxidant interaction. However, different patient categories may need different mixtures. Besides finding adequate research approaches, extensive costs will make the search for answers an interesting struggle.

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